STRUCTURAL STUDIES OF CHEMICAL CONSTITUENTS OF PLANTS OF BUNDELKHAND REGION

A THESIS

SUBMITTED FOR THE DEGREE OF
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By
(Km.) SHOBHA DEVI
M. Sc.

DEPARTMENT OF CHEMISTRY
DAYANAND VEDIC (P.G.) COLLEGE
ORAI-285001 (U.P.)
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CERTIFICATE

It is to certify that the thesis entitled, "STRUCTURAL STUDIES OF SOME CHEMICAL CONSTITUENTS OF PLANTS OF BUNDELKHAND REGION" submitted by (Km.) Shobha Devi fulfills all the requirements of Ph. D. degree of Bundelkhand University, Jhansi. She reported her own research work with the investigation of a new Polysaccharide, carried out under my supervision and guidance. She did her research work regularly and more than 200 days as desired by University Statute, at the research laboratory of Chemistry Department, Dayanand Vedic (Postgraduate) College, Orai of Bundelkhand University, JHANSI (U.P.).

(G. S. NIRANJAN)

January 28 ,1983.

Department of Chemistry,
D.V.(Postgraduate)College,
ORAI (JALAUN), U.P.
285 001.

PREFACE

The dissertation entitled, "STRUCTURAL STUDIES OF CHEMICAL CONSTITUENTS OF PLANTS OF BUNDELKHAND REGION", deals with the isolation and chemical examination of polysaccharide from the seeds of Zizyphus rugosa and fruits of Musasapientum, some constituents from the fruits of Gardenia gummifera. The thesis has been divided into four chapters.

The chapter I is of introductory nature and describes the wide importance of natural products and a brief account of different classes of compounds, i.e. polysaccharide, flavanoids and anthocyanins.

The chapter II deals with the isolation and structural elucidation of neutral water soluble polysaccharide from the seeds of Zizyphus rugosa.

The chapter III describes the isolation and structural elucidation of a water soluble neutral polysaccharide from the fruits of Musa sapientum.

The chapter IV is divided into three Sections (A), (B) and (C) deals with the isolation and elucidation of chemical structures of two flavonoids and a anthocyanin from the fruits of Gardenia gummifera.

A brief review of uptodate literature on chemical examination of selected plants, has been described respectively in each concerned chapters.

The work represented in the thesis has been carried out in the chemical laboratories of Dayanand Vedic Post-graduate College, ORAI, (Bundelkhand), under the supervision of Dr. G.S. Niranjan, D. Phil., F.I.C.S., Department of Chemistry, Dayanand Vedic (P.G) College, ORAI (Bundelkhand).

A brief summary of the entire work has been submitted separately alongwith the thesis, according to the requirement of ordinances for Ph. D. degree of Bundelkhand University.

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The achievement of the goal would have been a hard nut to crack without the constant encouragement and help given by Dr. Y.S. Srivastava, Dr. S.K.Katiyar, Dr. S.C. Sikroria and Dr. R.K. Gupta who provided valuable guidance, suggestions and inspiration during my research period.

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I will be failing in my duty if I do not record my sincere and heartfelt gratitudes to my parents and all family members for their co-operation, inspiration, guidance and zeal shown/given without whom the present important work could not have been accomplished successfully.

I also offer my extreme gratitude and most sincere thanks to Smt. Dr. G.S. Niranjan, for all the affections showred on me during the period.

Last but not least I also wish to express my profound gratitude and thanks to Sri A.K. Gupta, for his encouragement, co-operation and guidence given to me throughout the course of the research work which has been invaluable to me and which I can never forget.

CHEMICAL LABORATORIES,
D.V. (P.G.) COLLEGE,
ORAI - 285 001.

Shobha Devi
(SHOBHA DEVI)

CONTENTS

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CHAPTER - I

INTRODUCTION

There are still a large number of plants which have not been investigated for their active principles among the plant kingdom. The promiscous centre of nature awaits for plant chemist for their valuable investigations, may serve the human sufferings.

The plant products obtained from the plants are classified into following groups:(1) alkaloids, (2) glycoside, (3) saponins (4) terpenes and terpenoids, (5) bitter principles, (6) essential oils, (7) fatty oils and waxes, (8) lactones, (9) resins and tannins, (10) steroids and phyto steroides, (11) phenolic compounds, (12) organic acids, (13) hydrocarbons, (14) Gum and mucilage, (15) sugars.

This classification is not rigid, in the sense that one compound may be said to belong to more than one groups according to its molecular structure various chemical constituents obtained from the plants are classified into many groups. A brief account of the review on the classes of compounds investigated from the plants, which have been incorporated in the present thesis is given below :

I.2 - Polysaccharides.

I.3 - Flavonoids.

I.4 - Anthocyanin.

I.2 POLYSACCHARIDES

Polysaccharides are components of almost all living organism. They are most abundant in the higher orders of land plants and sea weeds where they constitute approximately three

quarters of the dry weight[5]. They are present in fungi exoskeleton of insect and crustaceans, in the capsules of micro-organism, in cartilage, in animal joint fluids etc.

Polysaccharides are macromolecular compounds composed of several monosaccharide units, usually linked through oxygen to give complex composition. They are regarded as condensation polymers of monosaccharides resulting from the formation of glycosidic linkage by elimation of water. They are hydrophillic colloids of high molecular weight, some completely soluble in water, other swell and absorb considerable amount of water without dissolving.

Gums and mucilages are complicated polysaccharide polymers and differ in the respect that the former are characterised as plants exudate-s while the latter are isolated from various plant organs by extraction with water. Plant gums and mucilages have been known and in use since very early times, reference being made to them in the 'Bible'; and they seem to ha ve been of commercial value for several thousand years, especially in India, Asia, Africa, Australia and China.

Polysaccharides act deserving roles in the physiology of plants, animals and microorganism as surface material and regarded as food reservers[6] in much the same manner as starch in many plants and glycogen in animals or as agent for holding water[6,7]. The plant is believed to synthesize the gum exudate in order to seal off the infected section of the plant and prevent further invasion of the tissue[8,9,10]. There is no concept as to the origin of gum exudates, whatever the exact origin and mode of formation of the gums may be, it is

reasonable to believe that gum exudates are formed by some type of enzymatic polymerisation and not by direct polymerisation.

Gums and mucilages are used in wide range of industries like cosmetics[11,12,13], pharmacy[14,15], textiles[16,17], adhesives[18], food products[19,20,21], paper[22,23], and in many other fields.

A polysaccharide is isolated from the plant by extraction with cold or hot water, water containing a little acetic acid and the precipitation of the soluble portion with the excess of ethanol. The polysaccharide is purified to remove the inorganic ions and proteinous impurities[24] by repeated precipitation with ethanol from acidified aquous solution.

The homogeneity of the polysaccharide is checked by fraction precipitation[25], zone electrophoresis[26,27] and acetylation and deacetylation[28]. A mixture of polysaccharide can be separated over a cellulose column[29,30], while the acidic polysaccharides may be fractionated as their complexes[31]. Ion exchange column[32] are also used effectively for the fractionation but the methylated gums are separated over alumina[33]. Electrophoretic separation of polysaccharides have been achieved mainly in borate buffer[34,35], but acetate buffer[36,37] and citrate buffer[37] have also been used. With the help of membranes or filters of desired porosity[38] polysaccharides may be fractionated. The unwanted polysaccharides of the mixture may be destroyed with specific enzymes[39] followed by denaturation of enzyme with heat, alkali and alcohal. The fractionation of polysaccharides may also be achieved by gel filtration[40] and molecular sieve[41].

The purified polysaccharide is subjected to preliminary determination of lignin, ash content, methoxyl, acetyl, primary hydroxyl and carbonyl groups and they are estimated after the detection of nitrogen, sulphur, phosphorus and halogens which may be present in the polysaccharide.

The optical rotation of the polysaccharide is measured by means of usual polarimeter[42] or photoelectric spectropolarimeters[43]. The configuration of glycosidic linkage in oligosaccharides can be correlated to optical rotatory power by applying Hudson's rule of isorotation[44].

The molecular weight of the polysaccharide having terminal reducing group can be determined by estimating it with C^{14} labelled sodium cyanide[45], sodium hypoiodite[46], ferricyanide[47], and periodate oxidation studies[48]. Physical methods like viscosity[49], light scattering[50], osmotic pressure[51] are also used to determined the molecular weight of the polysaccharide.

The hydrolysis of the polysaccharide with mineral acids under different conditions provides information regarding the nature of linkages present between sugar moieties. The complete acid hydrolysis of the polysaccharide results in the liberation of monosaccharides which can be separated by paper[52] or column chromatographic[53] techniques. They are identified by their R_f values, co-chromatography with authentic samples, melting points and by preparing their crystalline derivatives. Partial acidic hydrolysis with dilute mineral acids (0.01 - 0.1N) results in degradation of the polysaccharide into less complicated molecules which can easily be identified. Oligosaccharides, obtained by partial hydrolysis, can be separated by paper chromatography

and their structure is determined by the usual process of methylation, followed by the hydrolysis and identification of methylated sugars, periodate oxidation and enzymic hydrolysis. Enzymic degradation[54] provides various information about the polysaccharide. The sugar may be quantitatively estimated by microvolumetric method, spectrophotometric method or colorimetric method. Recently an extensive use of gas liquid partition chromatography[55,56] in the separation and estimation of sugars has been reported.

The polysaccharide is subjected to periodate oxidation to obtain the information regarding the nature of end groups and types of glycosidic linkage[57] present. It has been observed that the 1,2 -diol groups in 1→2 or 1 →4 linked and 1,2,3-triol groups in the 1 →6 linked anhydrohexose units are oxidised by one and two moles of periodate respectively, liberating one mole of formic acid but the units having 1 →3 linkages with no 1,2 - diol system are not effected. Thus by determining the consumption of periodate and amount of formic acid liberated, various informations regarding the structure may be obtained.

The methylation studies serve the valuable information regarding the types of linkages between sugar moieties in a polysaccharide. The method consists in the methylation of the polysaccharide followed by hydrolysis to give methylated sugars. The nature and the quantitative determination of the methylated sugars provide information on the relative proportions of non-reducing end groups, the degree of branching, the type of interchain linkages and the nature of the main chain linkages

in the polysaccharide. Methylation is usually carried out by means of Haworth's method[58] followed by Purdie's method[59]. The methylated product is hydrolysed in two steps, first the methanolic hydrogenchloride[60] or with 85 - 98% formic acid[61] and finally with the mineral acids. The methylated sugars are separated on paper and indentified by their R_{TMG}[62] values, optical rotations and melting points of their crystalline derivatives. The methylated sugars are quantitatively estimated by titrating them with alkaline hypoiodite or by colorimetric method. Those polysaccharides which are soluble in dimethyl sulphoxide, may be very efficiently methylated[63] in fewer steps by using methyl iodide and silver oxide.

In the present thesis, the chemical examination of two complex water soluble polysaccharides, first (isolated from the seeds of Zizyphus rugosa) and second (isolated from the fruits of Musa sapientum) have been described in chapter II and chapter III respectively.

I.3 FLAVONOIDS

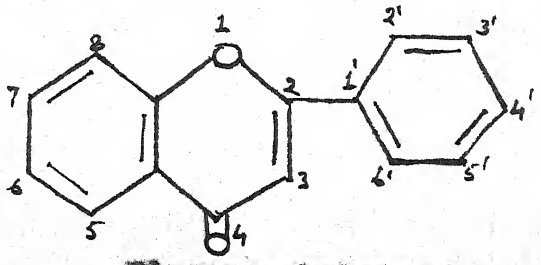
Flavonoids covers a largest group of naturally occuring oxyheterocyclic pigments. They includes chalcones, dihydrochalcones, aurones, flavones, flavonols, isoflavones, anthocyanins and leucoan-thocyanidins. In these two benzene ring are linked by a propane bridge ($C_6 - C - C - C - C_6$) except in isoflavones in which the structure based upon $C_6 - C - \underset{C_6}{C} - C$. Flavonoids are present in plants as yellow

pigments. These are found in the free state as well as in the form of glycosides, containing either sugars or more than one hydroxyl group or disaccharide (bioside) and tri-saccharides.

It is supposed that flavones protect plants from harmful ultraviolet radiations or from loss of important materials by autooxidation and one is tempted to believe physiological functions of the flavonoid pigments based upon their colours are related to the role of flowers in reproduction[64]. These compounds were found to be great medicinal importance as bacteriostatic[65] and insecticidal etc.

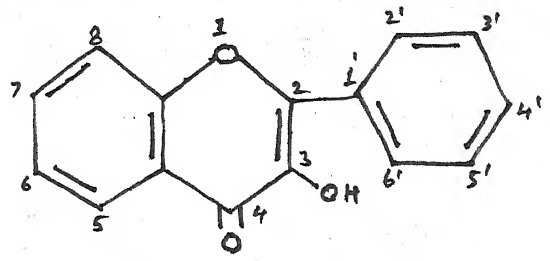
I.1.1 FLAVONES AND FLAVONOLS

The flavones and flavonols are naturally colouring matters. Their structure is based on that of 2 - Phenyl - 4 - Chromene. The basic unit of flavone and flavonols is γ- pyrone which is present as benzo - γ - pyrone (chromone). The benzo -γ- pyrone is substituted by a phenyl group in position - 2 to give the first member of the class of flavones when a hydrogen atom on C_3 in the γ-pyrone ring of flavone is replaced by a hydroxyl group, 3 - hydroxy flavone or flavonol is produced. The basic skeleton of flavone and flavonol may be represented as :



Flavone skeleton

The basic skeleton of flavonol may be represented as under :



Flavonol skeleton

The flavones which are also known as anthoxanthins are widely distributed yellow plant pigments. They occurs either in the free state or as glycosides or associated with tannins. They are also occur as colourless glycosides in the white corollas of several flowers which on treatment with ammonia vapour turns yellow (as the colourless precursors are converted into flavones).

Mostly, the flavones and flavonols occure as glycosides and on hydrolysis they yield the sugar moieties and a sugar free portion (a glycone) known as flavone or flavonol as the case may be. The position occupied by a sugar unit in glycosidic linkage, plays an important part due to which a glycoside exhibits difference in properties as solubility and capacity to form complexes with metals. Unlike anthocyanins in which the sugar residue is usually present at position 3 and 5, the sugar moiety in flavone and flavonols is generally attached to a hydroxyl group at position 3 or 7.

These compounds have been found to be highly physiologically active. The flavonol glycoside rutin has been described for its therapeutic properties. The insecticidal action of polyhydroxy flavones and their ethers and the action of

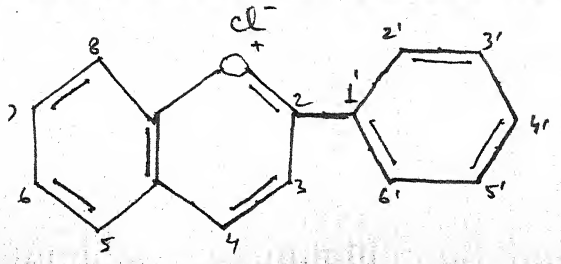
flavones on isolated enzyme system[66] have been studied.

The author has been able to isolate a flavone compound and a flavonol glycoside from the seeds of Gardenia gummifera. The chemical study of these colouring substances has been described in chapter IV of the thesis.

I.3.2. ANTHOCYANINS

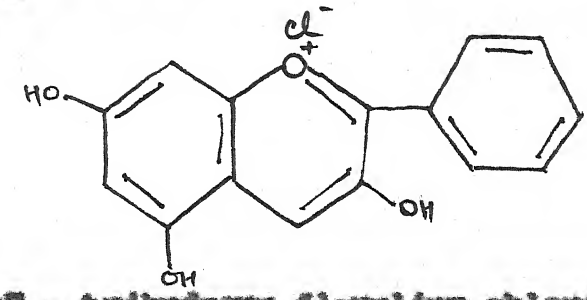
Anthocyanins comprises of a group of glycosidic pigments responsible for various colours, particularly red, violet, and blue, in flowers, fruits, (berries), stems, leaves and roots of the plants. They are soluble in water and generally occur in the aqueous cell-sap. Anthocyanins are amphoteric in nature, their acid salts are red, alkali, salts are blue and free anthocyanins (or neutral) are violet. The different shades of the flowers are due to the presence of some anthocyanins in different media (acidic, alkaline or neutral).

All the anthocyanins have flavylium chloride or 2 - Phenyl benzopyrylium chloride as the parent compound. Anthocyanins and anthocyanidines are derivatives of 3 : 5 : 7 - trihydroxy flavylium chloride. The various pigments (anthocyanins and anthocyanidins) are differ in the number, nature and position of other hydroxyl groups, methoxy groups and sugar residue. The basic skeleton of anthocyanidin is represented as follows :



Flavylium chloride

The basic skeleton of anthocyanidin is represented as under in continuation



3:5:7 - trihydroxy flavylium chloride

Many flowers that are first colourless, are known to develop colour rapidly and the colourless plant constituents which can be converted into anthocyanidins by boiling with aqueous or alcohlic hydrochloric acid, are known as leucoanthocyanins. Anthocyanins on hydrolysis with mineral acids or enzyme break into the sugar moiety and sugar free pigment, called anthocyanidins or aglycone. The common sugars found in anthocyanins are glucose, galactose, rhamnose.

The colour variation is due to the presence in the cell vacuole of a range of different anthocyanins. Flower colour varieties arise either by spontaneous generalation[67] within a single species or when two closely related species are hypridised.

The anthocyanins may be present in any part of the plant from the root tip to the flower stigma, but intense permanent pigmentation by anthocyanins are generally confined to petal or fruit tissue[68] . Deeply coloured flowers may be born on plants with essentially anthocyanin free stem and leaves.

REFERENCES

1. Stanl, E.; Chemiker. Ztg.. 82 , 323 (1958).
2. Fieser, M. and Fieser, L.R.; J. org. chem. , 13 800 (1948).
3. Dobriner, L.I. ; J. Amer. chem. SOC., 73, 3215 (1951).
4. Fuson, A. ; J. Amer. Chem SOC., 74 , 5206 (1952).
5. Royl. Whistler and Charles Smart; 'Polysaccharide Chemistry', P. No (1-18), (1953), New York.
6. Montemartini, L. ; Lavori ist. botan., 5 , 45 (1934) Palermo.
7. McNair, J.B.; Amer. J. Botany, 19, 168 (1932).
8. Frank, H.S. p Ann. Agronom., 17, 86 (1885) ; Brit. chem. Abstracts, 48 , 684 (1885).
9. Brooks, F.T. ; New Phytologist, 27 , 85 (1925).
10 Swarbrick, T. ; J. Pomology Hort. Sci. , 6 , 137, (1927).
11 Hilfer, H. ; Drug and Cosmetic Ind., 67 , 774 (1950).
12 Hedgrove, H.S. p Ind. Chemist. , 16 , 145 (1940).
13 Anderson, E. ; J. Chem. 2 , 853 (1932), Ed.
14 Schenk, G.; Med. Monatsschr., 3, 700 (1949) ; Chem. Abstr. , 44 , 1223 (1950).
15 Hanzlik, P.J. , De Eds, F. , Empey, L. W. and Farr., W.H. ; J. Pharmacol . , 32 , 273 (1927).
16 Pinel, A. ; Brit. Pat. , 522 , 815 (1940).
17 Herzog, R. O, and Meier, A. ; U.S, Pat. , 1 , 141, 545 (1915).

18. Oakman, A.E. ; Colloid. chem. , 6 , 248 (1946).

19. Parrin, F.M. ; Fr. Pat. , 860 , 210 (1941).

20. Pyenson, H. and Dahle, C.D. ; J. Dairy Sci. , 21, 169 (1938).

21. Stall, A.C. ; Food Research, 17 , 278 (1952).

22. Swanson, J.W. ; Tappi. 33 , 77, 451 (1950).

23. Ozawa, To ; J. Chem. Ind. 25 , 309 (1922) .(Japan).; chem. Abstr ; 16 , 4086 (1922).

24. Ball, D.J. and Young, F.G. ; Biochem. J. , 28 , 882 (1934).

25. O' Sullivan, C. ; J. chem. SOC., 45 , 41 (1884); 79 , 1169 (1901).

26. Joubert, F.J. ; J. South African Chem. Instt., 7 (2), 107 (1954).

27. Preece, I. A. and Hibkirk, R. ; chem and Ind. , 257 (1955).

28. Haworth, W.H. , Hirst, E.L. and Smith, F. ; J. chem. SOC. , 1914 (1939).

29. Amine, E.S. ; J. Chem. SOC. , 282 (1955).

30. Sarenson, G.S. ; Biochem. Biophys. Acta, 28 , 176 (1958).

31. Antonopoules, C.A. Borelius, E. Gadall, S., Hammostrom, B. and Scott, J.E. ; Biochem. Biophys. Acta, 54 , 123 (1961).

32. Neukom, H. , Deuel, H., Hari, W.J. and Kunding, W. ; Helv. chim. Acta, 43 , 64 (1960).

33. Jones, J.K. N. ; J. chem. SOC. , 333 (1944).

34. Foster, A.B. ; 'Advances in Carbohydrate chemistry', 12 , 81 (1957).

35. Fuller, K.W. and Northcote, D.H. ; Biochem. J. , 64 , 657 (1956).

36. Lewis , B.A. and Smith , F. ; J. Amer. chem. SOC. , 72 , 3929 (1957).

37. Brookhart, J.M. ; J. chromatog. , 20 , 191 (1965).

38. Mould , D.L. and Synge, R.L. ; Analyst. 77 , 964(1952).

39. Adams, M. ,Richtmyer, N.K. and Hudson, C.S., J. Amer. chem. SOC. , 65 , 136 (1943).

40. (a) Flodin, P. ; 'Dextran Gels and their applications in Gel filtration ' , Ph.D. , Dissertation, Upsala University, UPPSALA (1962).

(b) Nordin, P. ; Arch. Biochem. Biophys., 99 , 101 (1962).

41. (a) Flodin, P. and Porath, J. ; 'Chromatography', E. Heftman edition, Reinhold Pub. Corp., N.Y., P. 328 (1961).

(b) Jones, J.K. N. , Wall, R.A. and Pittal, A.O. ; Canad , J. chem. , 38 , 2285 (1960).

42. Kaemt, W. ; Starke, 14 , 246 (1962).

43. Klyne, W. ; 'Advances in Organic chemistry', 1 ,650 (1959).

44. Hudson, C.S. ; J. Amer. chem. SOC., 31 , 66 (1909).

45. Moyer, J.D. and Isbell, H.S.; Anal Chem., 30 1975 (1958).

46. Chanda, S.K. , Hirst, E.L. , Jones, J.K.N, and Percival, E. G.V.; J. chem. SOC. , 1289 (1950).

47. Nussenbaum, S. and Khasid, N.Z. ; Anal. chem., 24 501 (1952).

48. Whelan, W.J. ; 'Methods in Carbohydrate chemistry', Ed. by R.L. Whistler. 4 , 72 (1964).

49. Cowie, J.M.G. and Greenwood, C.T.; J. Amer. SOC., 2962 (1957).

50. Everett, W.W. and Foster, J. ; J. Amer. chem. SOC., 81 , 3459 (1959).

51. Jorgenson, B.B. and Jorgenson, O.B.; Acta. Chem. Scand., 14 , 213 (1960).

52 (a) Bourne, E.J., Lees, E.M. and Weigel, H.; J. Chromatog., 11 , 253 (1962).

(b) Hay, G.W. , Lewis, B.A. and Smith, F.J., Chromatog. 11 , 479 (1963).

53. Binkley, W.W. and Altenburg, W.F. ; Intern. Sugar. J. ; 66 , 217 (1964).

54. Araki, C. and Araki, K. ; Bull. chem. SOC. (Japan), 29 , 339 (1956) ; chem Abstr., 51 ,3465 (1957).

55.(i)Mc Innes, A. G., Ball, D.H., Cooper, F.P. and Bishop, C.T.; J. Chromatog., 1 , 556 (1958).

56.(ii)Bishop, C. T. and Copper, F. P. ; Canad. J. Chem., 38 , 388 (1960).

56. Kircher, H. W. ; Anal. Chem., 32 1103 (1960).

57. Hay, G. W. Lewis, B. A. and Smith, F. ; 'Methods in Carbohydrate Chemistry' , 1, 357 (1965).

58. Parikh, V. M., Ingle, T. R. and Bhide, B. V. ; J. Indian Chem. Soc., 35, 125 (1958).

59. Jones, J.K. N. ; J. Chem. Soc., 1055 (1947).

60. Hirst, E. L., Hough, L. and Jones, J. K. N. : J. C hem. Soc., 928 (1949).

61. Andrew, P. Hough, L. and Jones, J.K.N.: J. Chem. Soc., 3393 (1952) : Ibid., 2744 (1952).

62. Smith, F. and Montgomery, R. : 'The Chemistry of Plant Gums and Mucilages'. American Chemical Society Mono-graph series, Reinhold Pub. Corp., N.Y., p. 226 (1959).

63. Srivastava, H.C. : Tetrahedron Letters, 27 ,1869-73 (1963).

64. Blank, F. : Botan. Rev., 13 , 241 - 317 (1947).

65. Blank, F. : and Suter, R. : Experimentia , 4, 72-73 (1948).

66. Cuma-Coke, E. and Plaza Dales Reyes, M.:
(a) Bol. Soc. Biol. Santiago Chile, 4, 105-7 (1947).
(b) Bull. Soc. Chim. Biol., 29 573-82 (1947).

CHAPTER - II

A NEW WATER SOLUBLE NEUTRAL POLYSACCHARIDE FROM THE SEEDS OF ZIZYPHUS RUGOSA

XI.1. The present Chapter describes the isolation and structural elucidation of a water soluble neutral polysaccharide from the seeds of Zizyphus rugosa.

The plant Zizphus rugosa Lam. commonly known as 'Ber'. This plant belongs to the family Rhamnaceae[1], a straggling evergreen shrub often climbing or occasionally a small tree. Young branches, inflorescence, prickles and under side of the leaves usually clothed with dense rusty coloured tomentum. Prickles broad based, strong and hooked mostly solitary. Leaves variable, 2 - 5 inches in long, ovate, or elliptic from an oblique after cordate base. Main nerves are prominent. Flowers long peduncled, axillary and terminal cymes, forming on the usually leafless branches, long terminal panicles, calyx, pubescent, inside. Drupe ½ - ⅓ inches long, globose or oboroid, 1 celled, 1 - seeded with very thin crustacean stone. Flowering in March and April and fruit ripens during rainy season.

Indigenous and naturalized throughout India. Wild and cultivated in sub- Himalayan tract C & S. India to Ceylon, also in Burma, Dehradun, Bundelkhand, Rohilkhand, Gorakhpur.

The fruit is eaten and branches are lopped and fodder. Flowers used for the remedy in Menorrhagia throughout India[2]. The use of Egyption wood in ancient civilisation is reviewed, and the abilities of many these species to have resisted termite attack was studied. This specimen is 3000 - 4000 years old.

The oldest Egyptian wood belongs to the genus Zizyphus[3].

The work done in the past years on this genus was surveyed and the details of it are given in the tabular form.

II.2. The Brief Review of Chemical Examination of this plant in the Literature is Described as given below :

Genus	Plant species	Constituents	Parts	References
1. Zizyphus	-	Vitamin C Content	-	(1936)[4]
2. Zizyphus	-	Anthraquinone derivative	Wood	(1958)[3]
3. Jujuba (Florida grown)	-	Carotene and ascorbic acid content	-	(1948)[5]
4. Jujuba (Korea)	-	Lysine, aspartic acid, glycine, asparagine, glutamic acid & galactose	-	(1969)[6]
5. Zizyphus	Talani (Balkat) (Balco)	Lignin and -cellulose	Wood (Philippine)	(1932)[7]
6. Zizyphus	Spina Christi	Tannin	Bark	(1938)[8]
7. Zizyphus	-	Three Auxin Protectors	Stem gall tissue	(1969)[9]
8. Zizyphus	-	Spinisin and its acylated derivatives	Seeds	(1980)[10]
9. Zizyphus	Jaczerio	Detergent analysis	Bark	(1951)[11]
10. Zizyphus	Jaczerio	Jussic acid	Bark	(1957)[12]
11. Zizyphus	Xylophora	Betulinic acid	Bark & Wood	(1961)[13]
12. Zizyphus	Xylophyrus	Tannins & oleanolic acid	Fruits	(1963)[14]
13. Zizyphus	Xylophyrus	(-)-Leucoanthocyanin	Fruits	(1968)[15]

Genus	Plant species	Constituents	Parts	References
14. Zizyphus	Oenoplia	Two new basic Peptide Zizyphine I, Zizyphinine II, Butulinic acid, D-galactose, D-fructose, sucrose.	Root & Bark	(1963)[16]
15. Zizyphus	Oenoplia	Constitution of Zizyphinine	Root & Bark	(1969)[17]
16. Zizyphus	Mauritiana	Two peptide alkaloids Mauritine (A) & Mauritine (B)	-	(1972)[18]
17. Zizyphus	Mauritiana	Zizogenin (a new Sapogenin)	-	(1979)[19]
18. Zizyphus	Fructus	Water soluble carbohydrates, 36.1% fructose, 32.5% D-galactose, 14.8% Oligosaccharide, 1.4% arabinose, 2.5% galacturonan.	Fruits	(1969)[20]
19. Zizyphus	Fructus	Zizyphus saponins I, II, III & jujubaside B and jujubogenin	Fruits	(1981)[21]
20. Zizyphus	Nummularia	Na, K, Ca, Mg, Fe, Al, Cu, and Zn trace mineral constituents.	-	(1970)[22]
21. Zizyphus	Vulgaris	Fatty acid and Resin acids from ether extract.	Bark	(1934)[23]
22. Zizyphus	Vulgaris	Structure of Spinosin (flavone C glycoside)	-	(1979)[24]
23. Zizyphus	Vulgaris	Spinosin	-	(1979)[25]
24. Zizyphus	Vulgaris	A new saponin	-	(1981)[26]
25. Zizyphus	Vulgaris	Anaesthetics	Leaves seeds	(1941)[27] (1936)[28]
26. Zizyphus	Vulgaris	Chinese drug (extracted oil 89.16% fatty acids of which 90.75% are unsatd (Palmitic acid		

Genus	Plant species	Constituents	Parts	References
		& Phytosterol) including oleic, and β-Linoleic acids.		
27. Zizyphus	Vulgaris	Betulinic acid ($C_{30}H_{46}O_2$)	Seeds	(1946)[29]
28. Zizyphus	Jujuba	Leucocyanidin, Leucopelargonidin, Mesd and Betulinic & oleanothic acids	Bark &	(1961)[13]
29. Zizyphus	Jujuba	Ceryl alcohol, Alkaloids, Protopine and Berberine	Leaves	(1956)[30]
30. Zizyphus	Jujuba	Rutin	Leaves	(1968)[31]
31. Zizyphus	Jujuba	Five alkaloids of 13 membered cyclopeptide alkaloidal ring structure.	Leaves	(1978)[32]
32. Zizyphus	Jujuba	Tannins, anthraglucosides, & saponins, Alkaloids, coumarins, anthocyanins, flavone glucoside, and mucilage.	Fruits Leaves	(1968)[33]
33. Zizyphus	Jujuba	Carbohydrates, carotene, tannins, flavone glycosides, saponins, lipids, resins, and mucilage.	Fruits	(1969)[34]
34. Zizyphus	Jujuba	Cyclic adenosin monophosphate.	Fruits	(1980)[91]
35. Zizyphus	Jujuba	Oil, contained oleic, linoleic, arachidic and behenic acids.	Seeds	(1953)[35]
36. Zizyphus	Jujuba	Essential amino acid contents.	Seeds	(1969)[36]

Genus	Plant species	Constituents	Parts	References
37. Zizyphus	Jujuba	Sapogenin (Zeelin lactone)	Seeds	(1970)[37]
38. Zizyphus	Jujuba	Saponin (Juju beside S_1 structure elucidation by carbon-13 nuclear magnetic resonance)	Seeds	(1978)[38]
39. Zizyphus	Jujuba	Amines	Endosperms	(1969)[39]
40. Zizyphus	Rotundifolia	Oil content, protein and fatty acids	Seeds	(1979)[40]
41. Zizyphus	Sativa	Sativanine A & B (cyclopeptide alkaloids).	Bark	(1979)[41]
42. Zizyphus	Rugosa	Starch moisture 68.4%, ash content 1.65%, starch prepared 1.29%	Seeds	(1949)[42]

A number of chemical compounds have been already described in the above literature, but no attempt has been made for the isolation and structural elucidation of polysaccharides of Zizychus rugosa. Because of the medicinal and industrial values of the plant, it was considered worthwhile to isolate and establish the structure of the polysaccharide isolated from the seeds of Z. rugosa.

II.3. STRUCTURAL ELUCIDATION OF NEUTRAL WATER SOLUBLE POLYSACCHARIDE.

RESULTS AND DISCUSSION

The polysaccharide was isolated from the defatted seeds of Z. rugosa, extracted with water (1% acetic acid) and precipitated with ethanol. The polysaccharide was purified by repeated precipitation with ethanol to get a white fibrous mucilage with minimum ash content (0.8%). The homogeneity of the polysaccharide was checked by :

(i) Fractional precipitation.

(ii) Zone - electrophoresis.

(iii) Acetylation and deacetylation.

The polysaccharide was dissolved in water and separated into two fractions by fraction precipition with different volumes of ethanol. Both the samples were analysed quantitatively by the method of Hirst and Jones[43]. The results were essentially identical showing the homogeneity of the polysaccharide.

The polysaccharide was acetylated with acetic-anhydride by the usual method to give the acetylated product, $[\alpha]_D^{25}$ - 58° (in ethyl acetate, C. 1.2%). Deacetylation of the product gave a polysaccharide having the optical activity $[\alpha]_D^{25}$ - 105° (in water C. 0.8%). This confirmed the homogeneity of the polysaccharide as the original one has the optical activity $[\alpha]_D^{25}$ - 106°

(in water C. 0.5%).

Another portion of polysaccharide was separated by zone - electrophoresis in borate buffer (pH 9.3). The paper chromatogram was cut into 1.0 cm. segments, which were numbered consecutively from anodic end down to cathodic end. Each segment was eluted with distilled water, treated with phenol - sulphuric acid reagent and the absorbance of characteristic orange-yellow colour was measured in a Klett-Summerson photoelectric colorimeter, using filter No.50. A plot of the absorbance against segment number showed only a single sharp peak indicating the polysaccharide to be homogeneous.

The polysaccharide was slowly soluble in water, $[\alpha]_D^{25}$ - 106° (in water,C, 0.5 g per 100 ml. of solution), ash content 0.8%. The polysaccharide was found to be free of nitrogen, sulphur and halogens. The methoxyl,uronide and acetyl percentage were found to be negligible.

The complete acid hydrolysis of the polysaccharide with 2N-sulphuric acid followed by the paper chromatographic analysis of the hydrolysate revealed the presence of three sugars, D-galactose, D-xylose and L-arabinose. The identity of the sugars was confirmed by their specific optical rotations, preparation of their crystalline derivatives and co-chromatography with authentic samples.

The quantitative estimation of monosaccharide components by periodate oxidation, taking ribose as a reference sugar, showed that galactose, xylose and arabinose are present in the molar ratio 6:7:1 in the polysaccharide.

The graded hydrolysis of the polysaccharide with 0.05N sulphuric acid and subsequent paper chromatographic analysis of the hydrolysate, taken out at various intervals, revealed that galactose was liberated first followed by the liberation of D-xylose and L-arabinose respectively. This shows that most of the xylose and arabinose are linked together forming the backbone (main chain) of the polysaccharide and galactose units are linked as terminal groups.

The polysaccharide was methylated first by Haworth method using dimethyl sulphate and alkali[44] followed by Purdie's method[45] with methyl iodide and silver oxide. Thus partially methylated product $[\alpha]_D^{25}$ - 45° (in chloro-form C-1%), 0 CH_3. 33% to give a fully methylated product, $[\alpha]_D^{25}$ -40° (in chloroform, C,1.0%), OMe. 46.5%. The complete hydrolysis of the methylated polysaccharide and paper chromatographic analysis of the hydrolysate in solvent A, revealed the presence of six methylated sugars. The methylated sugars were separated on a preparative scale by chromatography on Whatman No.3 filter paper. The following methylated sugars were identified :

(I) 2,3,4,6 - tetra-O-methyl-D-galactose.

(II) 2,3,6 - tri-O-methyl - D-galactose.

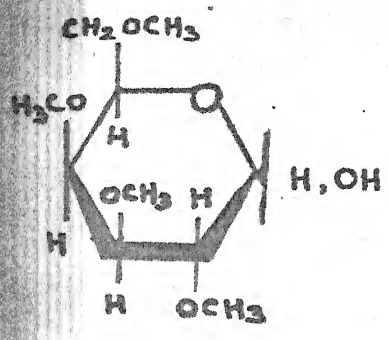
(III) 2,3,4 - tri-O-methyl- D-xylose.

(IV) 2,3 - di-O-methyl- D-xylose.

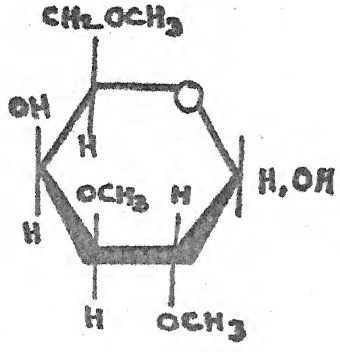
(V) 2, O-methyl - D-xylose.

(VI) 2 - O - methyl - L-Arabinose.

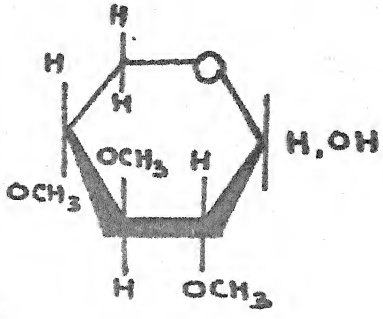
Methylated sugarI, had R_{TMG} in solvent A, 0.69.



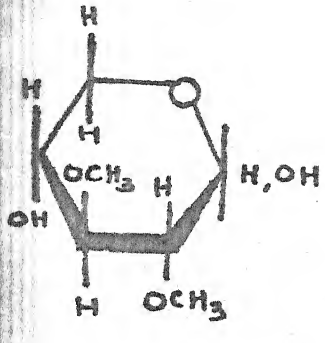
I



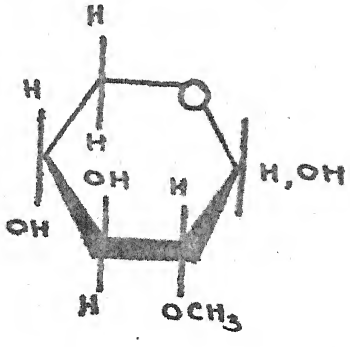
II



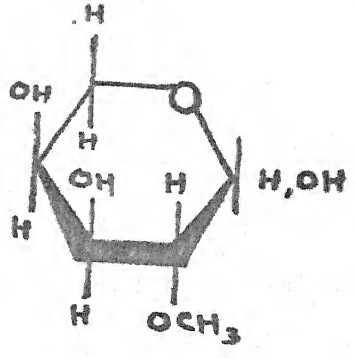
III



IV



V



VI

shows that the methylated sugar IV is, 2,3 -di-O-methyl-D-xylose.

Methylated sugar V, had R_{Xyl} value in solvent A 0.37, $[\alpha]_D^{25}$ - 25° (in water,C,2.3%), m.p. 132 -33°. It formed 2- O - methyl-D-xylose anilide, m.p. 122-24°, $[\alpha]_D^{25}$ + 214° (in ethyl-acetate, C, 0.8%). Its diacetate, 2-O-methyl, 3,4 - diacetate had m.p. 76-77°, $[\alpha]_D^{25}$ - 39° (in chloroform, C,2.8%). Thus the above observations confirmed that the methylated sugar V is 2-O-methyl-D-xylose.

Methylated sugar VI, had R_{Xyl} value in solvent (A), 0.11, $[\alpha]_D$ + 96° (in water, C, 0.8%) and in literature[49] is found $[\alpha]_D$ + 100°. It formed 2-O-methyl-N-phenyl glycosylamine when treated with ethanolic aniline, m.p. 140 - 142° [49a].

The quantitative estimation of methylated sugar, by the method of Hirst and Jones[50], showed that the sugars I, II, III, IV, V and VI were present in the molecular ratio 2:4:1:4:2:1.

The appearance of 2,3,4,6 -tetra-O-methyl-D-galactose I, and 2,3,4, tri-O-methyl-D-Xylose III, on hydrolysis of methylated polysaccharide indicates that (two) galactose units and xylose (1 unit) in the polysaccharide occupy terminal position as non-reducing end groups. The presence of 2,3 -di-O-methyl-D-xylose IV, (4 moles) and

2,3,6 -tri-O-methyl-D-galactose, II. (4 moles) indicates that the backbone of the polysaccharide consists of D-xylose and D-galactose units, through 1→4 linkages. detection of 2-O-methyl D-xylose, V, (2 moles) shows that xylose units in the main chain per repeating unit of the polysaccharide are linked at position -3 in addition to -1 and - 4. A single unit of the 2-O-methyl-L-arabinose, VI, indicates that L-arabinose is present in the centre of the polysaccharide and linked through, -1, -3 and -4 position.

Determination of terminal groups by periodate oxidation and subsequent titration of formic acid liberated corresponds to 0.1476 moles of formic acid per 100 g of the polysaccharide. On the basis of methylation studies, the simplest repeating unit of the polysaccharide, is supposed to consist of 14 sugar moities of which 2 units of galactose and one unit of xylose form terminal groups. considering such a repeating unit, the terminal groups were found 21.69% as determined by periodate oxidation studies, which is in close agreement to that revealed by methylation studies (21.42%).

During the periodate oxidation studies the oxidised polysaccharide was taken out from the reaction mixture after 60 hours and hydrolysed after destroying the periodate. The paper chromatographic examination of the hydrolysate showed that the presence of xylose was quite prominent

while no galactose could be detected. The paper chromatography of the hydrolysate of the oxidised polysaccharide taken out from the reaction mixture after 72 hours showed the presence of arabinose. The paper chromatographic analysis of the hydrolysate of the oxidised polysaccharide taken out from the reaction mixture after 84 hours showed the absence of all three sugars. It reveals that galactose units were completely oxidised within 60 hours, whereas xylose units were oxidised within 72 hours and arabinose was oxidised after 84 hours. The considerable difference in the rates of oxidation of the component sugars is due to steric effect resulting from the branched structure of the polysaccharide. The present knowledge, however, indicates that this phenomenon is most likely due to cyclic acetal formation[51].

The partial acid hydrolysis of the polysaccharide followed by paper chromatographic separation on preparative scale afforded six oligosaccharides which were detected as follows :

(1) 3^2-β-xylosyl xylobiose (O-β-D-xylopyranosyl (1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose.

(2) Rhodymenibiose (O-β-D-xylopyranosyl-(1→3)-O-β D-xylopyranose.

(3) Xylobiose (O-β-D-xylopiyranosyl-(1→4)-O-β-D-xylopyranose)

(4) D-galactopyranosyl-(1→4)-O-β-D-xylopyranose).

(5) 3-O-α-D-xylopyranosyl-L-arabinose

(6) 4-O-α-D- galactopyranosyl -D-galactose.

Oligosaccharide (1), m.p. 223°, $[\alpha]_D^{21}$ - 50° (in water, C, 2.7%), was chromatographically pure in solvent F and B. The molecular weight 420 corresponds to a trisaccharide of pentoses. Acid hydrolysis of the oligosaccharide yielded only xylose. The anomeric configuration of non-reducing xylose units were found to be 'β' by enzymatic hydrolysis and negative rotation. Partial acid hydrolysis yielded, xylobiose, rhodymanabiose, corresponding to oligosaccharide (3) and (2) respectively and xylose which were identified by co-chromatography with an authentic samples. Periodate oxidation studies showed the consumption of 4.3 moles of meta periodate with the liberation of 2.1 moles of formic acid. Hence the oligosaccharide was identified to be O-β-D-xylopyranosyl (1 → 3)-O-β-D-xylopyranosyl (1→4)- D-xylopyranose i.e. 3^2-β - xylosylxylobiose.(Fig. 1)

Oligosaccharide (2), a crystalline sugar, m.p. 191°, $[\alpha]_D^{22}$ -21° (in water, C, 2.91 %), was found to be chromatographically pure in two solvent systems F and B. The sugar on acid hydrolysis gave only xylose while the molecular weight of the sugar 296 corresponded to a pentose disaccharide. Enzymic hydrolysis with emulsin showed the presence of β -linkage between the two xylose units. The periodate oxidation showed the consumption of 3.26 moles of metaperiodate with the liberation of 1.18 moles of formic acid per mole of the sugar. The oligosaccharide is,

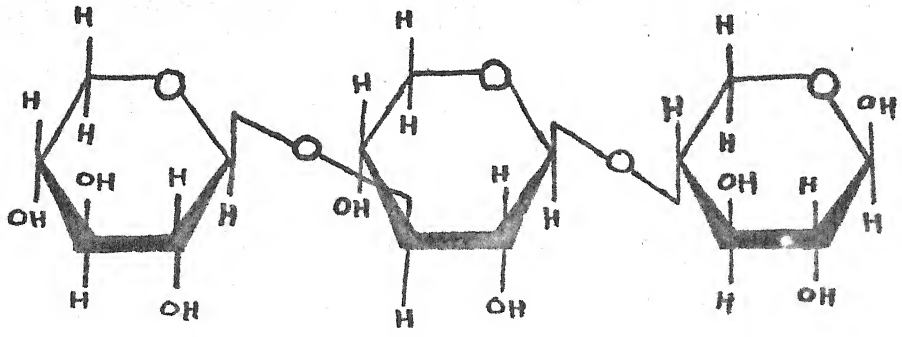


Fig - 1

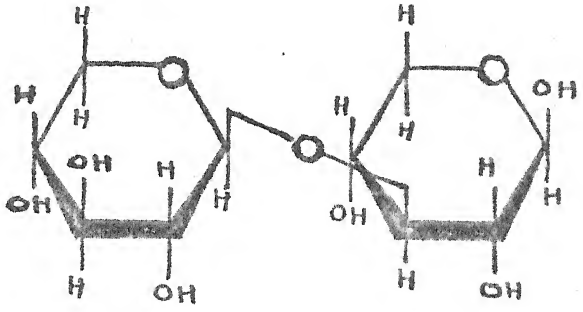


Fig - 2

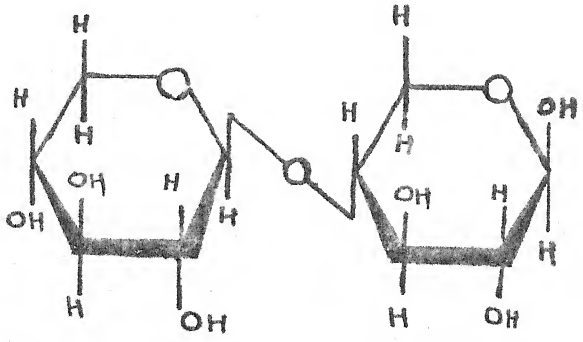


Fig - 3

therefore, identified to be rhodymenabiose (α-β-D-xylopyranosyl - (1→3) O - β - D-xylopyranose (Fig-2). The identity was confirmed by co-chromatography with an authentic sample.

Oligosaccharide (3), m.p. 187-88°, $[\alpha]_D^{20}$ -26.4° (in water, C, 3.5%), was chromatographically pure in solvent systems F and H. Acid hydrolysis showed the presence of xylose only. The molecular weight of the sugar was 298, corresponded to a disaccharide of xylose units. The periodate oxidation showed the liberation of 2.21 moles of formic acid with the consumption of 4.31 moles of metaperiodate per mole of the oligosaccharide. Hence the oligosaccharide was assigned the structure α-β-D-xylopyranosyl -(1→4) - xylopyranose.(Fig-3). The identity was further confirmed by co-chromatography with an authentic sample.

Oligosaccharide (4), m.p. 190-92°, $[\alpha]_D^{30}$ + 15° (in water), was shown to be chromatographically pure in solvent I. On acid hydrolysis revealed the presence of galactose and xylose units. The quantitative estimation by the method of Hirst and Jones[43] showed the molar ratio 1:1 between the two sugars in the oligosaccharide. The molecular weight 296, showed it, to be a disaccharide. Periodate oxidation studies afforded the liberation of 2.12 moles of formic acid and consumption of 4.14 moles of periodate per mole of the oligosaccharide.(Fig.4). Thus the sugar was confirmed to be D-galactopyranosyl -(1→4)-

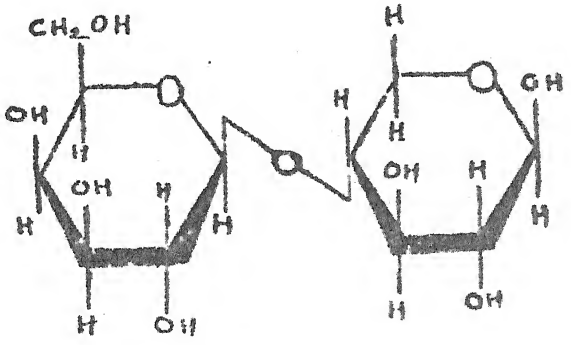


Fig-4

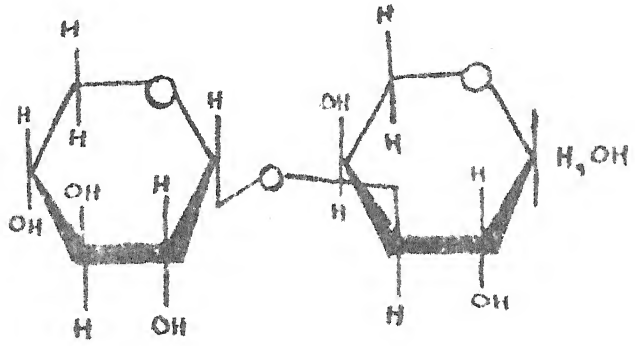


Fig-5

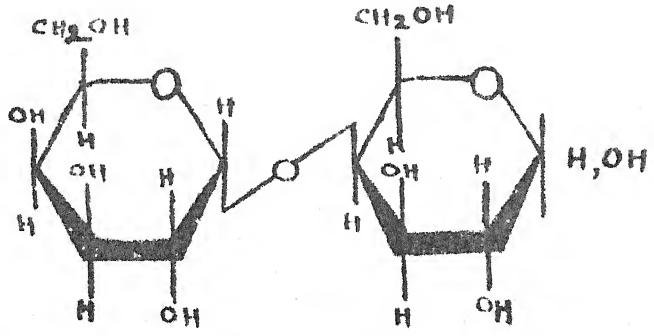


Fig-6

α -D-xylopyranose.

Oligosaccharide (5), syrup $[\alpha]_D^{25}$ + 172→180° (in water) R_g value in solvent R was found 0.48 chromatographically pure in solvent R. The complete acid hydrolysis followed by paper chromatographic analysis revealed the presence of two sugar & D-xylose and L-arabinose. The quantitative estimation by the method of Hirst and Jones[43] showed the molar ratio to be 1:1 between the two sugars in the oligosaccharide. Periodate oxidation studies showed the consumption of 3.25 moles of periodate with the liberation of 1.2 moles of formic acid. Methylation study of the oligosaccharide followed by acid hydrolysis of the fully methylated derivative afforded 2,3,4 -tri-O-methyl D-xylose and 2-O-methyl -L-arabinose in equal proportions. The oligosaccharide was not hydrolysed with emulsin indicating the α-linkage between the two sugars. The results proved that oligosaccharide was 3-O-α-D-xylopyranosyl-L-arabinose.

Oligosaccharide (6), was also a syrup. $[\alpha]_D^{25}$ +175° (in water C,1.2 %). The oligosaccharide was shown to be chromatographically pure in solvent-C. On acid hydrolysis followed by paper chromatographic analysis showed the presence D-galactose only. The molecular weight 367 calculated for $C_{12}H_{22}O_{11}$ 342 corresponded to a hexose disaccharide. Periodate oxidation studies showed the consumption of 4.22 moles of metaperiodate and liberation of 2.18 moles of formic acid. Methylation study of the oligosaccharide followed by acid hydrolysis it afforded

2,3,4,6-tetra-O-methyl-D-galactose and 2,3,6 -tri-O-methyl-D-galactose which was transformed by bromine water oxidation in alkaline solution afforded formaldehyde[64]. It was concluded that the biose linkage[65] was of 1→4 type. Oligosaccharide was not hydrolysed with emulsin it follows that the disaccharide must be 4-O-α-D-galactopyranosyl-D-galactose.(Fig. 6).

On the basis of the results obtained so far particularly from methylation studies, graded and partial hydrolysis. following valuable information could be derived :

(i) The main chain of the polysaccharide consists of β-(1→4) linkage and β-(1→3) linkage of xylose units and β-(1→4) linkage of xylose-galactose units alongwith β-(1→4) linkage of galactos and arabinose units.

(ii) Two units of galactose are linked as terminal groups in the main chain through β(1→4) linkages.

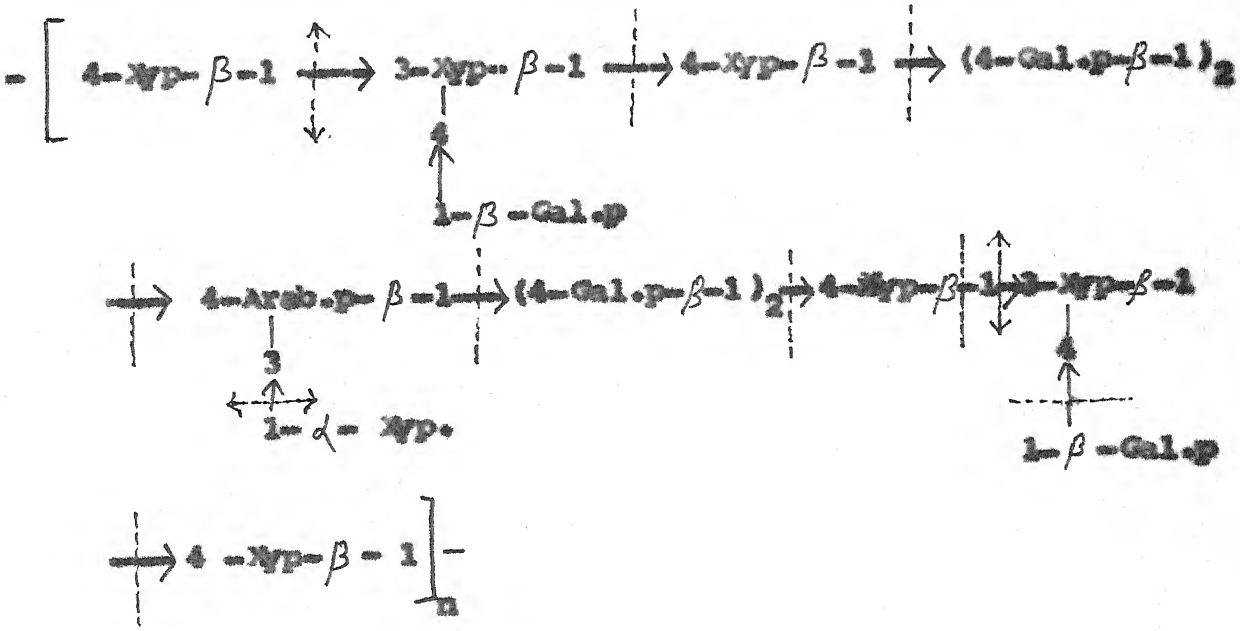
(iii) One xylose unit per repeating unit of the polysaccharide is linked through α-(1→3) linkage in the main chain as terminal group.

(iv) Only one unit of arabinose is linked through β-(1→4) linkage in the centre of the polysaccharide.

(v) From the above information, it is also clear that the galactose units in the side chain are linked at the same xylose units in the main chain

which linked through β (1→3) linkage in the main chain.

Taking all the experimental evidences into consideration togetherwith the structure of different oligosaccharide, the following most probable structure has been assigned to the polysaccharide from the seeds of Zizyphus rugosa.



Gal.p = D-galactopyranose

Arab.p = L-Arabinopyranose

Xyp = D-Xylopyranose

The structure contains 14 units of monosaccharide per repeating unit which fully explains the formation of oligosaccharide as obtained by partial hydrolysis and agrees well with the analytical data of the polysaccharide. The dotted and doubly arrowed dotted lines shows the probable

mode of fission of the linkages during the partial acid hydrolysis. The arrowed dotted lines indicated secondary hydrolysis.

The polysaccharide described above should consume 14 moles of metaperiodate with the liberation of 3 moles of formic acid per repeating unit of 14 sugar units. The actual consumption of periodate 14.08 and the liberation of formic acid 2.993 moles have been determined for per repeating unit of the polysaccharide, which are in close agreement to the calculated values.

Similar other structures may be possible but they are less probable because the formation of oligosaccharides as obtained in the present case might not be possible.

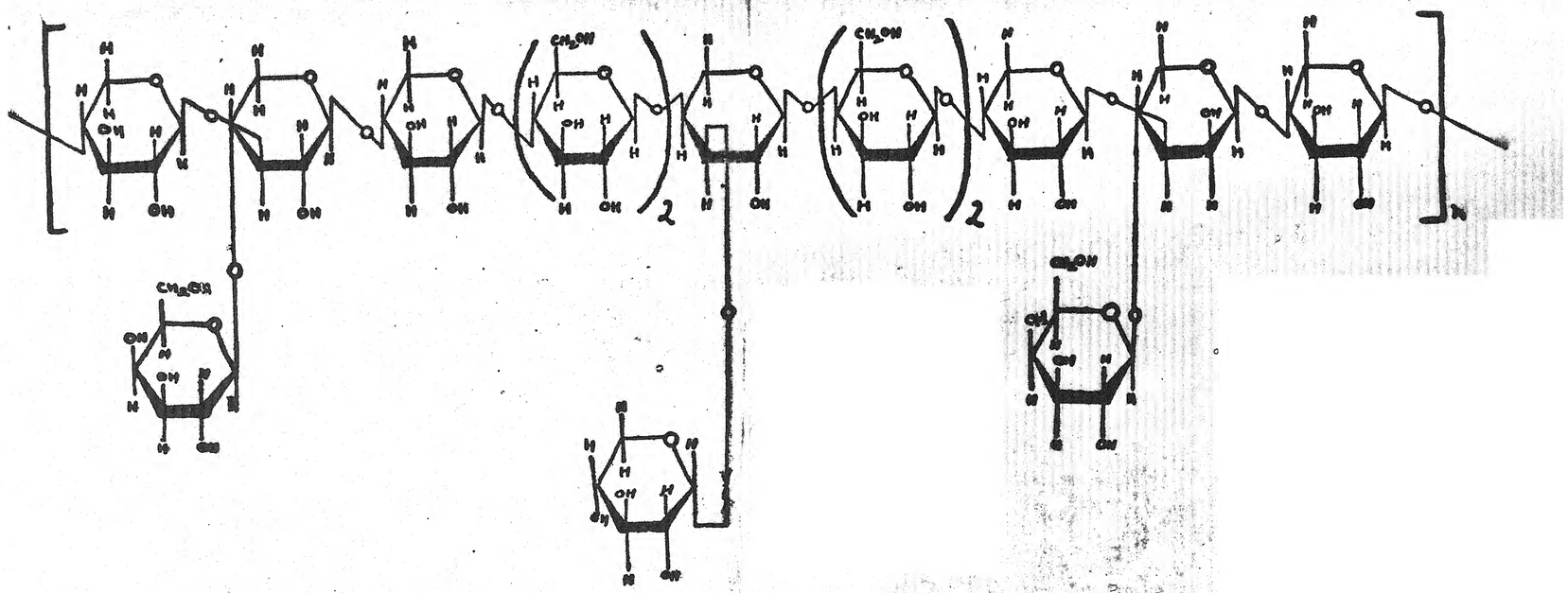
II. 4 EXPERIMENTAL

All evaporation were carried out under reduced pressure at low temperature unless specified otherwise. Residues were dried in vacuum at room temperature over anhydrous calcium choride. All specific rotations are inequilibrium values and all melting points are uncorrected. Paper chromatography was performed at room temperature by descending technique on Whatman No.1 filter paper unless stated otherwise, using following solvent system :

(A) n-Butanol - ethanol - water (4:1:5)[50]

(B) n-Butanol - acetic acid-water (4:1:5)[53]

(C) n-Butanol-isopropanol - water (11:6:3)[54]



STRUCTURE OF THE NEW POLYSACCHARIDE FROM THE SEEDS OF ZIZYPHUS RUGOSA

(D) Benzene - ethanol - water (169:47:15)[55]

(E) Butanone - water (11: 1)[56]

(F) Ethylacetate -pyridine - water (11:4:3)[57]

(G) Ethylacetate -pyridine - water (2:1:2)[58]

(H) n-Butanol - ethanol - water (40:10:19)[59]

(I) n-Butanol - ethanol - water (5:1:4)[60]

(R) Pyridine -ethylacetate - water (1:2.5:3.5)[61]

The spots were located by spraying a chromatogram with aniline hydrogen phthalate[62] and heating it at 110-120° for 10 - 15 minutes. Spectrophotometric determinations were carried out by a modification. of phenol - sulphuric acid method[63]. Klett-Summerson photoelectric colorimeter was used for measuring the absorbance.

II. 5 ISOLATION OF THE POLYSACCHARIDE

The dried and crushed seeds (2.0 Kg) were extracted successively with petroleum ether (60-80°) and ethanol. The extracted seeds were dried and then suspended in distilled water (2 litre) containing 1% acetic acid. The mixture was stirred mechanically for 8-10 hours to extract the mucilage as much as possible and squeezed out through a muslin cloth. The process was repeated six times when practically no precipitate was obtained by adding the extract to an excess of ethanol. The combined extracts were filtered thrice through a thick cotton pad, placed over a cloth in a Buchner funnel to remove the suspended fine particles. The clear mucilage solution so obtained was added slowly to a large excess of ethanol with constant vigorous stirring

when a fibrous colourless precipitate of the crude polysaccharide was obtained. It was filtered, washed with ethanol, followed by absolute ethanol and dried in vacuum at room temperature (4.5 gms ash 5.3%).

II.6 PURIFICATION

The dried crude polysaccharide was dissolved in distilled water (2 litres) containing 1% acetic acid with constant stirring. The solution was filtered and added very slowly to ethanol (8 litres) with constant and vigorous stirring and kept overnight. The precipitated polysaccharide was filtered and the above process was repeated thrice, to get a white fibrous mucilage (30 g ; ash 0.8 %).

II.7 HOMOGENEITY OF THE POLYSACCHARIDE

The homogeneity of the polysaccharide was checked by the following methods :

II.7.1 (a) Fractional precipitation

The pure mucilage (5 g) was dissolved in distilled water (500 ml). It was then added slowly to ethanol (500 ml) and the precipitated polysaccharide (Fraction I) was filtered, washed with ethanol followed by absolute ethanol and dried in vacuum. The filtrate was treated with another 1000 ml of ethanol with stirring and precipitated polysaccharide (Fraction II) was filtered, washed with ethanol and dried in vacuum. Both the fractions alongwith the original polysaccharide were hydrolysed separately with 2%

sulphuric acid. The sugar present in each hydrolysate were first identified by paper chromatography with authentic sugars using solvent (C) and then separated on two sheets of Whatman No.1 filter paper using the same solvent. The sugars were eluted with water and estimated quantitatively by periodate oxidation method[43]. The sugars eluted from one sheet were estimated by titration of formic acid liberated with standard alkali solution whereas the sugars from the other sheet were estimated by the method of consumption of periodate. The ratio of D-galactose, D-xylose and L-arabinose in both fraction was found almost the same (6:7:1), indicating the purified polysaccharide to be homogeneous.

II.7.2 (b) Acetylation and Deacetylation

The pure polysaccharide (3 g) was mixed thoroughly with anhydrous sodium acetate (10 g) and mixture was suspended in acetic anhydride (30 ml). After refluxing over a water-bath for 18 hours, the mixture was cooled to room temperature, and poured over crushed ice with constant stirring and then left overnight. The greyish white precipitate was filtered, washed with water and dried in vacuum. The dried mass was then dissolved in minimum quantity of acetone and the solution was poured slowly in distilled water, where upone a fine fibrous precipitate was obtained. This precipitate was filtered, washed and dried in vacuum 2.1 g. $[\alpha]_D^{25}$ - 58° (in ethyl acetate, C, 1.2%).

The dried acetylated polysaccharide (1.8 g) was dissolved in acetone (32 ml) and 50% potassium hydroxide

solution (32 ml) was added to it. The deacetylation was carried out in the usual manner[51] by refluxing the mixture over a water-bath for six hours. The viscous solution was poured slowly with stirring into 5% ethanolic acetic acid (300 ml) to precipitate the polysaccharide. The precipitate was filtered and was again precipitated by dissolving in water and dried. 0.59 g, $[\alpha]_D^{25}$ - 105° (in water, C, 0.8%).

The original polysaccharide $[\alpha]_D^{25}$ -106.5° (in water, C,0.5%) and the polysaccharide obtained after deacetylation had almost the identical specific rotation indicating the homogeneity of the polysaccharide.

II.7.3 (c) Zone - Electrophoresis

A strip support (15 cms x 45 cms) of Whatman No.1 filter paper was marked with a pencil in middle to indicate the starting line. 0.5% solution of polysaccharide (50 ml) was placed on starting line as a compact band. After drying at room temperature the strip was sprayed with borate buffer (pH 9.3) and suspended horizontally in the electrophoresis tank containing two electrode compartments each having aproximately 400 ml of borate buffer (pH 9.3). After electrophoresis at 260 V and 12.5 mA for 6.5 hours, the paper strip was dried. It was then cut lengthwise into 1 cm segments, which were numbered to the cathode end. The material from each numbered strip was eluted with water (6 ml) and filtered through glass wool. The filtrate (5 ml) was placed in a hard glass boiling tube with 8.5% aqueous phenol (1 ml). To the tube, concentrated sulphuric acid (15 ml) was added rapidly. The tubes were allowed to cool at

room temperature. The absorbance of characteristic yellow orange colour was measured in a Klett-Summerson photoelectric colourimeter using filter No.50. A blank was also run under the same conditions but without polysaccharide.

The reading so obtained were plotted against the segment number counted from the anode end to the cathod end. Only one sharp peak was obtained indicating the polysaccharide to be homogenous.

TABLE - I

Segment No.	Klett reading of elute	Blank Klett reading	Corrected Klutt reading	Absorbance
1	27	25	2.0	0.004
2	24	23	1.0	0.002
3	24	23	1.0	0.002
4	28	25	3.0	0.006
5	28	25	3.0	0.006
6	23	21	2.0	0.004
7	23	21	2.0	0.004
8	29	25	4.0	0.008
9	30	23	2.0	0.004
10	28	25	3.0	0.006
11	24	21	3.0	0.006
12	25	23	2.0	0.004
13	45	25	7.0	0.014
14	40	28	12.0	0.024
15	47	20	27.0	0.054
16	38	25	13.0	0.026
17	27	24	8.0	0.016
18	26	24	2.0	0.004
19	23	22	1.0	0.002
20	23	21	2.0	0.004
21	23	21	2.0	0.004
22	25	21	4.0	0.008
23	24	21	3.0	0.006
24	27	25	2.0	0.004
25	26	27	1.0	0.002
26	28	26	2.0	0.004
27	27	24	3.0	0.006
28	26	25	1.0	0.002
29	27	25	2.0	0.004
30	28	26	2.0	0.004

Absorbance was measured on 5 ml portion of coloured solution.

$$\text{Absorbance} = \frac{2 \times \text{Klett reading}}{1000} .$$

II.8 ASH CONTENT

The dried polysaccharide (0.3g) was ignited in a silica crucible previously heated to a constant weight. After ignition the crucible was cooled in a desiccator and weighted. From the weight of residue (0.0010 g), the ash content was calculated to be 0.8%.

II.9 PHYSICAL AND CHEMICAL EXAMINATION

It was a fibrous white powdered, very light in weight, slowly soluble in water. $[\alpha]_D^{25} - 106.5^\circ$ (in water, C, 0.5 g per 100 ml of solution). For the purpose of optical rotation, the solution was filtered through a sintered funnel to get a clear solution and the amount of polysaccharide in the solution was determined colorimetrically. The polysaccharide was found to be free of nitrogen, sulpher and halogens. It did not reduce Fehling's solution.

II.10 EXAMINATION OF FREE SUGARS

The polysaccharide was examined for free sugars by applying three spots of its solution in water on a strip of Whatman No.1 filter paper (15 cms x 45 cms). The paper was developed in solvent (A) for 36 hours, dried and cut lengthwise into three strips, each containing one spot. The three strips were sprayed with three different reagents using naphthoresorcinol and trichloroacetic acid (gives colour with ketoses only)[66] on one, aniline hydrogen

phthalate[62] on the second and silver nitrate in acetone followed by ethanolic sodium hydroxide[67] on the third. The first two paper dried in the oven at 120° and the third was air-dried. None of the strip showed any spots hence the polysaccharide did not contain any free sugar.

II.11 METHOXYL GROUP DETERMINATION

The percentage of methoxyl groups was determined by the method of Belcher, Fildes and Nutten[68] and was found to be 0.31%.

II. 12 ACETYL GROUPS DETERMINATION

The method by Belcher and Gobert[69] was followed for the determination of acetyl group percentage with and without mucilage found acetyl 0.90%.

II. 13 URONOID CONTENTS DETERMINATION

The uronoid contents were found to be negligible by the semi-micro method of Barker, Foster, Siddiqui and Stacey[70].

II. 14 HYDROLYSIS OF POLYSACCHARIDE AND DETERMINATION OF MONOSACCHARIDES

The purified mucilage (1.5 g) was dissolved in 2N sulphuric acid (100 ml) and was hydrolysed on a water-bath for about 24 hours. The hydrolysate was neutralised with barium-carbonate, filtered and concentrated under reduced pressure. The hydrolysate was examined for monosaccharide as described below :

II.14 (a) Paper chromatography

The spots of the hydrolysate were applied on two sheets of Whatman No.1 filter paper. The papers were developed separately in solvents (A) and (B) by descending unidimensional technique. The chromatograms were air-dried and sprayed with aniline hydrogen phthalate. On heating them in an oven at 120°, each chromatogram showed three spots. The R_f and R_G values of the three spots corresponded to D-galactose, D-xylose and L-arabinose as given in the following table.

TABLE - 2

Sugar identified	Solvent (A)		Solvent (B)	
	R_G found	R_G 71 given	R_f found	R_f 53 given
D-galactose	0.06	0.07	0.16	0.16
D-xylose	0.16	0.15	0.29	0.28
L-Arabinose	0.11	0.12	0.20	0.21

G = 2,3,4, 6-Tetra-O-methyl-D-glucose.

The identity of the three sugars was further confirmed by co-chromatography with an authentic samples of the sugars.

II. 14 (b) Column Chromatography

A portion of hydrolysate was dissolved in a small amount of aqueous methanol (1:1) and absorbed over a well washed column of cellulose(1" x 15"). The column was left overnight and the separation was effected with solvent (A) and several fractions (15 ml) each were collected. Such

fraction was analysed by paper chromatography with authentic samples of D-galactose, D-xylose and L-arabinose in solvent (B). The fraction 1-10 containing same sugar were combined together and concentrated to give D-xylose. It was recrystallised from aqueous methanol. $[\alpha]_D^{30}$ + 17.7° (in water, C, 1.15%). The melting point of the sugar was found to be 142-44°. The following derivative was prepared.

D-Xylose Phenyl Osazone Derivative

The osazone of the sugar was prepared by heating (250 mg) of sugar, 50 mg of phenyl hydrazine hydrochloride and 0.3 g of sodium acetate dissolved in 5 ml of water in a test tube and heated for 30 minutes on a boiling water-bath. Precipitate of the osazone started appearing after 7 minutes. The flocculent precipitate was separated with water, recrystallised from 50% ethanol, m.p. 161° resembling to an authentic sample.

The fraction 15-30 containing same sugar were mixed and concentrated to give D-galactose. It was recrystallised from aqueous methanol. $[\alpha]_D^{25}$ +78.5° (in water, C, 0.6%). The melting point of the sugar was found to be 167°. The following derivatives were prepared.

(i) D-Galactose Phenyl Hydrazone

Found	Given (Lit.)[72]
m.p. 152-53°	154-55°

(ii) N-p-Nitrophenyl-D-Galactosylamine

In a microtest-tube galactose (25 mg), p-nitroaniline (25 mg), a drop of glacial acetic acid and 2 drops of methanol : water(8:1 v/v) were taken. The whole mixture was boiled for

8 minutes and kept overnight in a refrigerator. The crystalline product was filtered, washed with cold ethanol, ether and dried in vacuum. It melted at 217-18° after recrystallisation from methanol. Lit.[73] m.p. 219°.

The fraction 33-40 were mixed and concentrated to give L-arabinose. It was recrystallised from aqueous methanol. $[\alpha]_D^{29}$ + 104 (in water, C, 1.26) m.p. and m.m.p. 155° in literature[74], it was found $[\alpha]_D^{29}$ + 101° , m.p. 150°. The following derivative was prepared.

L-arabinose phenyl osazone derivative

The sugar (0.2g) gave phenyl osazone on heating with phenyl hydrazine hydrochloride (0.4 g), crystalline sodium acetate (0.6 g) and water (6 ml) on a boiling water-bath for 30 minutes. The solution was cooled and the precipitate phenyl osazone was filtered and recrystallised from aqueous ethanol, m.p. and m.m.p. with an authentic sample 163-64° [75].

II.14 (c) Thin-layer Chromatography

The plates were prepared from slurry of silic gel-G in 0.1N solution of boric acid and the spots of hydrolysate alongwith benzene : acetic acid : methanol (1:1:3)[76] and airdried. These plates were sprayed with aniline hydrogen phthalate reagent. On heating them at 120° in an oven, three spots corresponded to D-galactose, D-xylose and L-arabinose were observed.

II.15 QUANTITATIVE ESTIMATION OF MONOSACCHARIDE

The method due to Hirst and Jones[43] was applied for quantitative estimation of component sugars of the

polysaccharide.

The polysaccharide (300 mg) was dissolved in 2N sulphuric acid (20 ml) in a 250 ml round bottom flask. The flask was then heated for 24 hours on a water-bath. After cooling to room temperature the hydrolysate was diluted to 30 ml and then D-ribose (20 mg) was added to it. The whole solution was shaken well and transferred to the beaker. The solution was neutralised with barium carbonate and filtered. The filtrate and the washing of the barium carbonate were concentrated and then made upto 10 ml.

Six sheets (30x45 cms) of Whatman No.1 filter paper were used as paper chromatography. Three guide strips(4x45cms) two on either edges and one in centre, were marked on each paper. A portion of above solution was placed along the starting line (8 cms away from the upper edge) of the three sheets, whereas the remaining three sheets were used as blanks. A guide spot was placed in the centre of each guide strips. All the sheets were developed in solvent (C) for 48 hours. After drying the chromatograms, guide strips were cut lengthwise, sprayed with aniline hydrogen phthalate and heated in an oven at 120° to locate the position of sugars. With the help of guide strips, appropriate sections of unsprayed portion were cut alongwith the blank strips of same dimension from the blank chromatograms. Each section (with and without sugar) was cut into small pieces and extracted separately with 10 ml of hot water. The eluted sugars were then oxidised with 0.25M sodium metaperiodate(5ml). The liberated formic acid was titrated with standard alkali, after destroying the excess of metaperiodate with ethylene glycol (2 ml), using methyl red as

indicator. Blank readings were substracted to get the titre values.

TABLE - 1

Sugar	Value of alkali* used (in ml)			Corresponding amount of sugar(in mg)		
	A	B	C	A	B	C
Galactose	9.34	7.80	10.52	2.756	2.300	3.104
Xylose	10.48	8.74	11.80	3.221	2.686	3.627
Arabinose	1.50	1.24	1.68	0.461	0.381	0.516

* Strength of NaOH = $\frac{N}{122}$

Assuming complete recovery of D-ribose, the above results indicate that in the polysaccharide D-galactose, D-xylose and L-Arabinose are in the molar ratio of 6:7:1.

II. 16 GRADED HYDROLYSIS[77] OF THE POLYSACCHARIDE

The polysaccharide (200 mg) was dissolved well in 0.05N sulphuric acid (20 ml) and the hydrolysis was carried out over a boiling water-bath. The hydrolysates were taken out at various intervals, and examined chromatographically without removal of sulphuric acid using solvent (B) for the purpose of irrigation of the paper. Results are given in table -4.

TABLE - 4

Time (in minutes)	Sugar identified	No. of other spots
5	Galactose (Faint)	
10	Galactose (Faint)	
15	Galactose + xylose(Faint)	
20	Same as above	
30	Same as above	
60	Same as above	Two spots
90	Same as above	Two spots
120	Galactose + xylose + Arabinose(Very Faint)	Three spots
180	Galactose + xylose + Arabinose	Four spots
240	Same as above	Same as above
420	Same as above	Same as above

During graded hydrolysis of the polysaccharide galactose was found to be liberated first followed by xylose and then arabinose. The earliest release of D-galactose and simultaneously of D-xylose and L-arabinose (Faint) leads to the consumption of D-galactose are present as terminal groups and some units of D-xylose are also present as terminal groups instead of main chain of the polysaccharide. As galactose is liberated earlier than xylose, this is most probably attached to the main chain by more easily hydrolysable linkage. Arabinose is liberated after 120 minutes indicating that L-arabinose is present as main chain of the polysaccharide and this is most probably attached in the main chain by hardly hydrolysable linkage.

XI.17 METHYLATION OF POLYSACCHARIDE

The polysaccharide was methylated first by the method of Parikh, Ingle and Shide[44] followed by Purdie's method[45].

The polysaccharide (6.8 g) was dissolved in minimum amount of water and then taken in a conical flask fitted with B.^{24} joint. Dimethyl sulphate (40 ml) and 40 % sodium hydroxide (80 ml) were added dropwise with constant stirring by magnetic stirrer. The temperature was maintained between 40-50°. After repetition of the above procedure, the solution was concentrated under reduced pressure and filtered to remove the sodium sulphate. The filtrate was again concentrated to a thick syrup and dissolved in acetone. This was then methylated by repeating the above procedure thrice. The finally concentrated solution was extracted thoroughly with chloroform. The extracts were dried over anhydrous sodium sulphate and the solvent distilled off under reduced pressure. The partly methylated product was brownish mass. (5.9 g), $-OCH_3$, 33%, $[\alpha]_D^{25}$ -45° (in chloroform,C,1 per 100 ml of solution).

The partly methylated polysaccharide was further methylated by Purdie's method[45]. The partly methylated polysaccharide (5.6 g) was dissolved in methanol (36 ml) in a conical flask fitted with three necked multiple adapter. The temperature was maintained at 40-50° by placing the conical flask, fitted with air-condenser having fused $CaCl_2$-tubes in a through containing water over the magnetic stirrer. Methyl iodide (9 g) and silver oxide (6 g) were added with continuous stirring in several equal instalments, each after half an hour interval. After the final addition the reaction mixture was heated for four hours on a water-bath under reflux and then filtered after cooling the contents. The silver salts were exhaustively extracted with chloroform

under reflux. The combined filtrate and extracts were evaporated under reduced pressure and the resulting syrup was remethylated thrice under the same conditions. The fully methylated polysaccharide was obtained as a deep brown coloured product. (4.8 g) $-OCH_3$, 46.5 %, $[\alpha]_D^{25}$ -40° (in chloroform, C, 1.0%).

II.18 HYDROLYSIS OF THE METHYLATED POLYSACCHARIDE AND IDENTIFICATION OF METHYLATED SUGARS

The hydrolysis of the methylated polysaccharide was carried by slight modification of method due to Souveng et al[78]. The methylated polysaccharide (100 mg) was dissolved in 85% formic acid (20 ml) and solution was refluxed for 4 hours on a water-bath. The solution was then cooled and concentrated under reduced pressure and traces of formic acid were removed under vacuum. It was dissolved in 0.25N sulphuric acid(10 ml) and the hydrolysis was carried out for 16 hours on a water-bath. The hydrolysate was cooled, neutralised with barium carbonate and filtered. The residue was washed with water followed by ethanol. The combined solutions were concentrated under reduced pressure to light brown syrup. The methylated sugars were separated on Whatman No.1 filter paper using solvent (A). The chromatograms showed six spots after spraying with aniline hydrogen phthalate and drying at 120°. The R_{TMG} (TMG = 2,3,4,6-Tetra-O-methyle -D-glucose) value of each methylated sugars was calculated in solvent(A). These values were compared with that given in literature as shown in the following table.

TABLE - 5

Methylated sugars identified	Solvent(A)	
	R_{TMG} found	R_{TMG} 50,79 given
2,3,4,6-Tetra-O-methyl-D-galactose	0.89	0.88
2,3,6-Tri-O-methyl-D-galactose	0.70	0.71
2,3,4-tri-O-methyl-D-xylose	0.95	0.94
2,3,-di-O-methyl-D-xylose	0.75	0.74
2,0 -methyl-D-xylose	0.37	0.38
2,O-methyl-L-arabinose	0.11	0.12

II.19 QUANTITATIVE ESTIMATION OF METHYLATED SUGARS

The methylated polysaccharide (200 mg) was hydrolysed as described above. After hydrolysis, glucose(60 mg) was added to hydrolysate. It was then neutralised with barium carbonate and filtered. The residue was washed with ethanol. The filtrate and washing were concentrated under reduced pressure to a syrup. A portion of the syrup was dissolved in acetone and applied on three sheets (A,B, and C) of Whatman No.1 filter paper. Each having three guide strips. The papers were irrigated with solvent(D) alongwith three blank sheets. After development of chromatograms and locating the sugars on guide strips, appropriate sections, containing sugars were cut from the unsprayed portion of the chromatograms. The sugars were eluted with 10 ml of water.

The methylated sugars were estimated by alkaline hypoiodite method[50]. The eluted portions were taken in 50ml conical flasks separately provided with ground glass joint stoppers, and a solution (2 ml) containing 0.2M sodium bi-carbonate and 0.2M sodium carbonate was added solution of

iodine (0.1N, 2 ml) was then added to the reaction mixture and the flask was stoppered. The experiments as corresponding blank elutes were also carried out in the same way. After three hours, the reaction mixture was acidified cautiously with 2N sulphuric acid and 15% potassium iodide solution(2ml) was then added to it. The liberated iodine was titrated against 0.01N sodium thiosulphate solution using starch as indicator. The results are given in table-6.

TABLE - 6

Fraction & sugar		Volume of 0.01N hypo used (in ml)			Corresponding amount of sugar (in mg)		
		A	B	C	A	B	C
A	2,3,4,6-tetra-O-methyl-D-galactose	2.02	2.06	2.40	2.201	2.245	2.616
B	2,3,6-tri-O-methyl-D-galactose	4.32	4.40	5.16	4.406	4.496	5.218
C	2,3,4-tri-O-methyl-D-xylose	1.26	1.30	1.50	1.096	1.131	1.305
D	2,3-di-O-methyl-D-xylose	5.52	5.62	6.52	4.416	4.496	5.216
E	2-O-methyl-D-xylose	3.02	3.08	3.60	2.204	2.248	2.628
F	2-O-methyl-L-arabinose	1.50	1.54	1.80	1.095	1.124	1.314
G	D-Glucose	3.42	3.50	4.08	3.078	3.150	3.672

The above results correspond to an average molar ratio between A:B:C:D:E:F as 2:4:1:6:2:1. The methylated sugars were calculated as the methyl ethers of anhydrohexose and anhydro-pentose i.e. $C_6H_{12}O_5$, $C_7H_{14}O_5$ and $C_8H_{16}O_5$ for mono-, di-, and tri- O-methyl-D-xylose respectively and $C_9H_{18}O_6$ and $C_{10}H_{20}O_6$ for tri-, and Tetra-O-methyl-D-galactose respectively and $C_6H_{12}O_5$ for mono-O-methyl-L-arabinose. An average recovery of the methylated polysaccharide was found

to be 90.90% assuming 100% recovery of D-glucose.

II.20 CHARACTERISATION OF METHYLATED SUGARS

The methylated polysaccharide was hydrolysed according to the method of Garegg and Lindberg[80]. Methylated polysaccharide (4.0g) was dissolved in 72% sulphuric acid (50 ml). The solution was kept for one hour at room temperature(25°) and then diluted to 200 ml. Further hydrolysis was carried out by heating for 4 hours on a water-bath. The solution was cooled neutralised with barium carbonate and filtered. The residue was washed with water followed by ethanol. The solutions were concentrated to a syrup under reduced pressure.

The mixture, containing different methylated sugars, was resolved into six fractions on Whatman No.3 filter paper using solvent (D). Strips, containing different individual methylated sugars, were eluted with water. The elutes were concentrated separately under reduced pressure and marked as fractions I, II, III, IV, V and VI.

II. 20.5. Fraction I

A solid, R_{TMG} in solvent (A) 0.89, found OMe, 51.5% calculated for tetramethyl hexose. OMe 52.4%, $[\alpha]_D^{25}$ + 123°, (in water, C.0.5%). Lit[86,87,88] for 2,3,4, 6-tetra-O-methyl-D-galactose $[\alpha]_D^{16}$ + 142° → + 117° (equil.)(in water C,1.1%), m.p. 71-73°. It gave a red colour with p -anisidine hydrochloride spray in Butanol and a brownish red colour with aniline hydrogen phthalate. On treatment with ethanolic aniline gave 2,3,4,6 -tetra-O-methyl-N-phenyl-D-galactosylamine, m.p. 189-90°. $[\alpha]_D^{25}$ - 80° (in acetone,C, 1.1%) Lit[86] m.p. 193-94°

Lit[69] m.p. 192° $[\alpha]_D$ - 77°. Therefore, the identity of methylated sugar is established as 2,3,4,6-tetra-O-methyl-D-galactose.

II.20.2 Fraction XI

Solid, m.p. 96°, R_{TMG} in solvent (A) 0.70, $[\alpha]_D^{25}$ -40 → - 36° in water, Lit[47], m.p. 98°, R_{TMG} in solvent (A) 0.70 in Lit. $[\alpha]_D$ - 44 → -37° It formed 2,3,6-tri-O-methyl amide with the treatment of concentrated ammonia solution, m.p. 134°. Lit[46]. m.p. 135°. It formed 2,3,6-tri-O-methyl phenyl hydrazide with phenyl hydrazine hydrochloride having m.p.173° Lit[48]. 175°.

II.20.3 Fraction XII

Syrup, it could not be recrystallised. The R_{TMG} in solvent (A) 0.95, optical rotation of sugar was found to be $[\alpha]_D^{18}$ + 19.2 (in water, C,0.35%). Lit[83] is $[\alpha]_D^{15}$ + 20.3° . One found, 55.15% , calculated for $C_8H_{16}O_5$ is 55.35 %.

The anilide of the sugar was prepared by refluxing the dry syrup (38 mg) with freshly distilled dry aniline (120 mg) for three hours in a water-bath (85-95°) in absolute ethanolic solution (5 ml). Ethanol was distilled off and the whole viscous mass was kept in the refrigerator for seven days. The 2,3,4-tri-O-methyl-D-xylophranosyl anilide failed to crystallise. It came out as a white powder by the addition of 3-4 drops of dry acetone. The precipitate was filtered out and dried, (yield 10 mg). The m.p. of powder was found to be 94-95°. $[\alpha]_D^{22}$ -83° (in ethanol,C,2.5%) Lit[83]. m.p. 120°,$[\alpha]_D$ - 84 → + 47° and Lit[20]. m.p. 91°.

The methoxyl value of the derived anilide was found to be 33.5% ($C_{14}H_{21}O_4N$ requires-OMe, 34.8%).

The sugar in this fraction was thus identified as 2,3,4-tri-O-methyl-D-xylose.

II.20.4 Fraction XV

Syrup, R_{TMG} in solvent (A) 0.7?, found OMe, 34.8%, dimethyl xylose, $C_7H_{14}O_5$, requires-OCH_3, 34.8%. The optical rotation of the sugar $[\alpha]_D^{20}$ + 22.5 (in water, C,4.6%). Lit[83]. $[\alpha]_D^{15}$ + 23°.

The anilide of the sugar prepared by the method of Hampton[84]. The dry syrup (200 mg) were refluxed for six hours with 1.5 ml of freshly prepared distilled dry aniline dissolved in 10 ml of absolute ethanol. The ethanol was distilled off and the bulk of the aniline was removed under high vacuum, (5-6 mm of mercury) at 65-70° (bath temperature). The syrup mass was kept in the refrigerator for 72 hours, when tiny crystals (plates) were observed. The adhering aniline was removed by the addition of dry ether, and the crude light brown crystals was filtered out, washed with ether, and dried. (yield 38 mg), m.p. 137-38° for 2,3, di-O-methyl-D-xylopyranosyl anilide in Lit[83]. m.p. is 145° and optical rotation $[\alpha]_D^{21}$ + 192° (in ethyl acetate C,0.3%) and in Lit[85] $[\alpha]_D$ +185° (in ethyl acetate).

The methoxyl content of 2,3-di-O-methyl-D-xylopyranose anilide (recrystallised was found to be 25.2% calculated for $C_{13}H_{19}O_4N$. OMe, 24.8%). The sugar present in this fraction was identified as 2,3-di-O-methyl-D-xylose.

II.20.5 Fraction V

Solid, R_{TMG} in solvent (A) 0.37, OMe, 18.94% calculated for mono methyl pentose, $C_6H_{12}O_5$, OMe, 18.90% , m.p. 132-33°. $[\alpha]_D^{25}$ - 25° (in water C,2.3%). Lit[81] m.p. 135-37°, $[\alpha]_D$ - 23 → + 35° (in water). Lit[82] m.p. 132-33°. $[\alpha]_D$ - 24 → + 36° (in water). It formed 2-O-methyl-D-xylose anilide on treatment with ethanolic aniline, m.p. 122-24° , $[\alpha]_D^{25}$ + 214° (in ethyl acetate, C, 0.8%). Lit[81], m.p. 125-26° , $[\alpha]_D$ + 214° (in ethyl acetate).

On acetylation of sugar with anhydrous sodium acetate and acetic anhydride a greyish white precipitate was obtained. The dried mass dissolved in minimum quantity of acetone and the solution was poured slowly in distilled water, where upon a white crystalline compound 2-O-methyl-D-xylose, 3,4-diacetate, m.p. 76-77°, $[\alpha]_D^{25}$ - 3.9° (in chloroform, C,2.8%). Lit[82] m.p. 78-79°, $[\alpha]_D$ - 38° (in chloroform).

II.20.6 Fraction VI

Syrup, R_{TMG} in solvent (A) 0.11 in Lit[49]. 0.12. , $[\alpha]_D$ + 96°(in water, C, 0.8%). Lit.[48,49] $[\alpha]_D$ + 100° (in water). It formed 2-O-methyl-N-phenyl glycosylamine when treated with ethanolic aniline having m.p. 140-42° in Lit.[49(a)] m.p. 142°.

II.21 PERIODATE OXIDATION OF THE POLYSACCHARIDE

II.21(a) Liberation of formic acid[83] and estimation of end group

The polysaccharide (500 mg) was dissolved in water

(5 ml) and in this solution, potassium chloride(0.5 g) and 0.25-M sodium metaperiodate (60 ml) were added. The volume was made upto 140 ml with water. In a blank experiments, potassium chloride (0.5 g) and (0.25 M) sodium metaperiodate (60 ml) were diluted to 140 ml with distilled water. The oxidation was carried out in a dark at room temperature, 5 ml of aliqu were drawn at various intervals alongwith blank and excess of metaperiodate was reduced with 2 ml of ethylene glycol. The liberated formic acid was titrated against N/110 sodium hydroxide using methyl red as indicator. Results are given in table -7.

TABLE - 7

Time (in hours)	Reading with blanks (in ml)	Volume of alkali used (in ml)	Corresponding amount of formic acid liberated in mg	Total formic acid liberated in mg
8	0.0	1.12	0.4683	13.11
16	0.0	1.20	0.5018	14.05
24	0.0	1.34	0.5603	15.69
36	0.0	1.46	0.6105	17.09
48	0.0	1.60	0.6690	18.73
60	0.0	1.70	0.7109	19.90
72	0.0	1.74	0.7276	20.37
84	0.0	1.74	0.7276	20.37

The data shows that 0.1476 moles formic acid was liberated (72 hours) per 100 gm of the polysaccharide. The amount of formic acid liberated (72 hours) corresponds to 21.69 % of anhydrohexose and pentose units present as end groups. The titre value of alkali at 48,60, and 72 hours indicated that one mole of formic acid liberated per 736.91g. 693.48g and 677.5g of the polysaccharide respectively.

After 72 hours, 25 ml portion of reaction mixture was taken out, acidified with 2N sulphuric acid (5 ml) and then 10% potassium iodide (4 ml) was added to it. The liberated iodine was titrated immediately against 1N sodium thiosulphate solution without using starch as indicator till the solution become colourless. The solution was concentrated to 10 ml to which 2N sulphuric acid (10 ml) was added and the hydrolysis was carried out for 16 hours on a water-bath. The hydrolysate was neutralised with barium carbonate, filtered and the filtrate was concentrated to a syrup under reduced pressure. The syrup was examined by paper chromatography using different solvents the chromatograms revealed the presence of arabinose.

II.21(b) Consumption of Metaperiodate[92]

The polysaccharide (300 mg) was dissolved in water (70 ml) to which 0.25 M sodium metaperiodate (40 ml) was added and the total value of it was made upto 120 ml with water. A blank was also prepared with 0.25 M sodium metaperiodate (40 ml) diluted to 120 ml with water. The periodate oxidation was carried out at room temperature. 2.0 ml aliquots were withdrawn from the reaction mixture and blank at various intervals and 30% potassium iodide solution(2 ml) was added followed by addition of 0.5N sulpuric acid (3 ml). The liberated iodine was titrated immediately against 0.0404 N sodium thiosulphate solution using starch as indicator. The reading with the polysaccharide were substracted from the corresponding reading of control experiment to get the titre values. The results are given in the table - 8.

TABLE - 8

Time (in hour)	Volume of hypo used (in ml)	Corresponding amount of periodate consumed (in mg)	Total periodate consumed (in mg)
8	1.18	5.100	306.01
16	1.32	5.705	342.32
24	1.42	6.137	360.25
36	1.50	6.483	389.00
48	1.60	6.915	414.94
60	1.68	7.261	435.68
72	1.72	7.434	446.06
84	1.72	7.434	446.06

The amount of metaperiodate consumed (72 hours) corresponds to the consumption of 0.6947 moles periodate per 100 g of polysaccharide. After 72 hours periodate oxidised solution (10 ml) was hydrolysed with 2N sulphuric acid. The hydrolysate was examined chromatographically for the presence of D-galactose, D-xylose and L-arabinose. The chromatogram showed the absence of all the three sugars.

II. 22 PARTIAL ACID HYDROLYSIS OF POLYSACCHARIDE

The polysaccharide (7 g) was suspended in water (500 ml) in a three necked flask and was dissolved stirring mechanically. The hydrolysis was carried for four hours at 80° by adding 0.2N hydrochloric acid (5 ml) and the solution was stirred throughout the process. The contents, after cooling down at room temperature were poured in ethanol (2 litres) to precipitate the degraded polysaccharide. The precipitate was filtered and washed well with ethanol. The filtrate and washing were neutrilised with silver carbonate

with stirring. The precipitate was filtered, washed with water and combined solutions were concentrated under reduced pressure to a syrup.

II. 22.1 Examination of the precipitate

The precipitate was hydrolysed with 2N sulphuric acid for 18 hours, over a water-bath. The hydrolysate was cooled, neutralised with barium carbonate and filtered. The filtrate and washings were concentrated and examined chromatographically over Whatman No.1 filter paper using solvents (A) and (C). The chromatograms showed three spots corresponding to R_g values of D-galactose, D-xylose and L-arabinose which was confirmed by co-chromatography with their authentic samples. Due to small amount of precipitate, further studies were not possible.

II.22.2 Examination of the hydrolysate

The hydrolysate was examined paper chromatographically using solvents (A),(B),(C) and (G). The chromatograms showed seven spots on spraying with aniline hydrogen phthalate and drying at 120°, indicating the presence of seven sugars.

II.22.3 Separation of Oligosaccharides

The syrup was dissolved in minimum quantity of water and applied on twenty sheets of Whatman No.3 paper as long thin band, three inches below the upper end and one inch away from the outer edges. Each paper has three guide strips, two on outer edges and one in the centre. After developing the paper on solvent (B), for sixty hours, they

were dried. The guide strips were cut from the chromatograms, sprayed with aniline hydrogen phthalate and dried at 120° with the help of the guide strips appropriate sections were cut from the unsprayed portion of the chromatograms and sugars were eluted with water. In all, seven fractions were obtained.

II.22.4 Examination of Fraction I and identification of 2-O-β-D-xylosylxylobiose (O-β-D-xylopyranosyl(1→3)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose).

This fraction was crystallised from ethanol, m.p. 223° $[\alpha]_D^{21}$ - 50° (in water, C, 2.7%). Xylotriose values were 1.38 and 1.45 in solvents (F) and (B). R_x values in solvent (F) and (B) were found 0.73 and 0.25 respectively.

The complete acid hydrolysis with 2N sulphuric acid subsequent neutralisation with barium carbonate and examination by paper chromatography indicated the presence of xylose only, which was further confirmed by co-chromatography with an authentic sample. The molecular weight of the sugar was found to be 420 by hypoiodite method[50] which corresponds to trisaccharide of pentose units. Molecular weight calculated for $C_{15}H_{26}O_{13}$, 414.

Partial acid hydrolysis of trisaccharide with 0.5N hydrochloric acid at 100° for 30° minutes gave xylose, xylobiose and rhodymanbiose. Periodate oxidation studies revealed that one mole of the pligosaccharide consumed 4.3 moles of metaperiodate and 2.1 of formic acid liberated. It also confirmed the presence of 1- 3 linkage between two

xylose units in oligosaccharide molecule.

The sugar was completly hydrolysed with emulsin, suggesting the presence of β - linkage. From the above observations the sugar was identified to be -O-β-D-xylopyranosyl-(1→3)-O-β-D-xylopyranosyl (1→4)-D-xylopyranose i.e. 3^2-β-l-xylosylxylobiose. The constants of sugar are given below in table -9.

TABLE - 9

Constants	Found	Reported	References
m.p.	223°	225°	(93)
Optical rotation	$[\alpha]_D^{21}$ -50°	$[\alpha]_D^{22}$ -52° - -47 ± 1°	(93)
R_x xylotriose in solvents(F) &(B)	1.38 & 1.45	1.36 & 1.43	(93)
R_x values in solvents(F) &(B)	0.73, 0.25	0.72, 0.20	(93)

II.22.5 Examination of Fraction II and identification of Rhodymenabiose

R_x xylobiose values were 1.97 and 1.02 in solvents(B) and (F) respectively. Recrystalised from methanol, m.p. 198°, $[\alpha]_D^{22}$ - 21° (in water,C,2.91%).

Acid hydrolysis of the sugar with 2N sulphuric acid and neutralisation of the hydrolysate with barium carbonate followed by paper chromatography in solvent (C), reveals that the presence of xylose only. The molecular weight was determined by hypoiodite method[50], 296, molecular weight calculated for xylobiose, $C_{10}H_{18}O_9$, 282.

The periodate oxidation studies showed the consumption of 3.24 moles of metaperiodate with the liberation of 1.18 moles of formic acid. The sugar was completely hydrolysed with emulsin, showing the presence of β-linkage. The identity was further confirmed by preparing its phenyl osazone derivative, m.p. 198-199°, $[\alpha]_D^{22}$ + 47° (in pyridine, C, 2.2%) , and calculated for $C_{22}H_{28}O_7N_4$, N, 12.18 % found 12.30 % constants of sugar were compared with those reported in literature shown in table 10.

TABLE - 10

Sugar or Derivative	Constants	Found	Reported	Reference
Rhodymanobiose	m.p.	191°	192-93°	(94)
Rholymanobiose	$R_{xylobiose}$ in solvent (B)	1.97	1.97	(95)
-do-	Optical rotation	$[\alpha]_D^{22}$ -21°	$[\alpha]_D^{22}$ - 18. 4 $\pm$ 0.6°	(95)
3-O- β-D-xylopyranosyl-D-Xylose-phenyl osazone	m.p.	198-99°	194-96°	(94)
-do-	Optical rotation	$[\alpha]_D^{22}$ +47°	$[\alpha]_D$ +47°	(94)

II.22.6 Examination of fraction III and identification of Xylobiose

The fraction was recrystallised from aqueous ethanol, m.p. 187-98°, $[\alpha]_D^{20}$ - 26.4° (in water, C, 3.5%). R_x values were in solvents (B) and (F) 0.30 and 0.86 respectively.

Hydrolysis of the sugar with 2N sulphuric acid and neutralisation of the hydrolysate with barium carbonate

followed by paper chromatography in solvent (C), revealed the presence of xylose only which was further confirmed by co-chromatography with an authentic sample. The molecular weight of the sugar was 298, calculated for $C_{10}H_{18}O_9$, 282.

The periodate oxidation of sugar consumed 4.31 moles of metaperiodate with the liberation of 2.21 moles of formic acid indicating the (1→4) linkage between xylose units. The oligosaccharide completely hydrolysed with emulsin indicating the β-linkage between two units.

Thus the oligosaccharide is a disaccharide composed of D-xylose linked through -β-glycosidic bond. The sugar was identified 4-O-β-D-xylopyranosyl-D-xylose, which was confirmed by preparing the phenyl osazone derivative, m.p. 205° and $[\alpha]_D^{25}$ - 52° (in pyridine : ethanol).

The constants of sugar are given in table - 11.

TABLE - 11

Sugar or derivative	Constants	Found	Reported	References
Xylobiose	m.p.	187-88°	185°, 187°	(88)
-do-	Optical rotation	$[\alpha]_D^{20}$ -26.4°	$[\alpha]_D^{20}$ -25°, $[\alpha]_D^{20}$ -32° → → 25.5°	(96) (98.97)
-do-	R_x in solvent(B)	0.30	0.33	(96)
Phenyl osazone	m.p.	205°	205°	(96)
-do-	Optical rotation	$[\alpha]_D^{25}$ -52°	$[\alpha]_D$ -50°	(96)

II.22.7 Examination of Fraction IV and identification of 4-O-β-D-Galactopyranosyl(1→4)-D-Xylopyranose[52,96].

The fraction was recrystalised from methanol having the optical rotation $[\alpha]_D^{30}$ + 1.5° (in water) m.p. 190-92°. Acid hydrolysis with 2% sulphuric acid and neutralisation of the hydrolysate with barium carbonate, followed by paper chromatography, revealed the presence of D-galactose and D-xylose. The quantitative estimation by the method of Hirst and Jones[43] showed the molar ratio 1:1 between two sugars in the oligosaccharide.

Periodate oxidation studies showed the consumption of 4.35 moles of periodate and liberated 2.1 moles of formic acid.

Methylation of the disaccharide and followed by acid hydrolysis of the fully methylated derivative afforded 2,3,4,6, tetra-O-methyl D-galactose and 2,3-di-O-methyl D-xylose in equal proportions. The oligosaccharide was completely hydrolysed with emulsin indicating the presence of β-linkage between the two units. These results proved that oligosaccharide was 4-O-β-D-galactopyranosyl-D-xylopyranose.

II.22.8 Examination of Fraction V and identification of 3-O-α-D-xylopyranosyl-L-arabinose.

Syrup. $[\alpha]_D^{25}$ + 172-180° (in water). R_x in solvent (R) 0.48. Molecular weight of the sugar was 298. calculated for $C_{10}H_{18}O_9$,282. The sugar was hydrolysed with 2% sulphuric acid and neutralised the hydrolysate with barium carbonate.

followed by paper chromatography revealed the presence of D-xylose and L-arabinose. The quantitative estimation by the method due to Hirst and Jones[43] showed the molar ratio between two sugars in the oligosaccharide to be 1:1.

Periodate oxidation studies showed the consumption of 3.25 moles of periodate with the liberation of 1.2 moles of formic acid.

Methylation studies of the oligosaccharide followed by acid hydrolysis of the fully methylated derivative of oligosaccharide afforded 2,3,4-tri-methyl-D-xylose and 3-O-methyl L-arabinose in equal proportions. The oligosaccharide was not hydrolysed with emulsin indicating the presence of linkage between the two units. The results proved that oligosaccharide was 3-O-α-D-xylopyranosyl- L-arabinose.

The constants of sugar are given in table-12.

TABLE - 12

Syrup of sugar	Found	Reported	References
R_x in solvent(R)	0.48	0.49	(61)
Optical rotation	$[\alpha]_D^{25}$ +172° $\longrightarrow$ $\longrightarrow$ 180°	$[\alpha]_D^{25}$ +175° $\longrightarrow$ $\longrightarrow$ 180°	(61)

II.22.9 Examination of Fraction VI and identification of 4-O-α-D-Galactopyranosyl-D-galactose

Syrup, $[\alpha]_D^{25}$ + 175° (in water, C,1.2%). Lit[65] $[\alpha]_D$ +177° (in water).

The sugar was hydrolysed with 2N sulphuric acid, followed by neutralisation of hydrolysate with barium -

carbonate and paper chromatography, revealed the presence of D-galactose units only. The molecular weight was found to be 347 calculated for $C_{12}H_{22}O_{11}$, 342 by hypoiodite method, which corresponded to a disaccharide of hexose units.

Periodate oxidation studies showed the consumption of 4.22 moles of metaperiodate with the liberation of 2.18 moles of formic acid.

Methylation study of the oligosaccharide followed by acid hydrolysis of fully methylated oligosaccharide afforded 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,6-tri-O-methyl-D-galactose which was transformed by bromine oxidation in alkaline solution afforded formaldehyde[64]. It was concluded that the biose linkage was 1→4 type. Oligosaccharide was not hydrolysed by hydrolysis with emulsin, it follows that the linkage was α-type.

The above observations identified the oligosaccharide to be 4-O-α-D-galactopyranosyl-D-galactose.

<u>XI. 22.10 Examination of Fraction VII and identification of L-arabinose.</u>

M.P. and m.m.p., 156°, $[\alpha]_D^{29}$ + 104° (in water, C, 1.26%) Lit[74]. m.p. 150°, $[\alpha]_D^{29}$ + 101°.

The sugar (.2 gm) gave phenyl osazone on heating with phenyl hydrazine hydrochloride (0.4 gm), crystalline sodium acetate (0.6 gm) and water(6 ml) on a boiling water-bath for 30 minutes. The solution was cooled and the precipitated phenyl osazone was filtered and recrystallised from aqueous ethanol, m.p. and m.m.p. with an authentic sample 163°-164° [75].

REFERENCES

1. Duthie, J.F.; 'Flora of the Upper Gangetic Plain'. Vol. 1 P. 154, (1960). Copyright by the Government of India.
2. Chopra, R.N., I.Chopra, I.C., and Nayar, S.L.; Glossary of Indian Medicinal plants', page No: 262,(1956)
3. Sandermann,W., Dietrichs, H.H. and Gottwald, A. ; HolzRoh-4, Harz-steff. 16 , 197 -204 (1958).
4. Chakrabarty, P.K. ; Indian J. Med. Research, 23 , 247-51(1935).
5. French, R.B. and Abbott, C.D. , Florida Agr. Expt. Sta. Techn. Bull. , 444 , 21, (1948).
6. Back, K.W., Lee, S.Y., Han, D.S. , Kim, J.J. ; Yon' gu Nonmunjip, Chunchon Nonghoe Taehak, 3 , 21-4 (1969).
7. Yenko, E.M.; Baena, L., West, Augustus, P. and Curran. H.M.; Phillippine J., Sci., 47 , 343-48 (1932).
8. Simoncini, E.; Boll. Staz. Sper. Ind. pell Mat. conciantii., 16 , 173-82 , (1938).
9. Tandon, P.; Arya, H.C. ; Experientia, 36 (8) , 958-9, (Engg.) (1980)
10. Woo, Wan Sick; Shin, Mak Hyung; Kang, Sam Sik; Recent. Adv. Nat. Prod. Res. Proc. Int. Symp., (Pub. 1980) 33-40 (Eng.) (1979).
11. Rosa, J.S. and Ichan, A.; Anais Assoc. quim. Brasil., 10 , 236-53 (1951).
12. Antonaccio, L.D. ; Rev. Quim. Ind. (Rioda Janeiro). 26 , 126 (1957).
13. Harbhajan Singh, Seshadri, T.R. and Subramanian,G.B.V.;

Curr. Sci. 34. (11). 344-45 (1961).

14. Rajadurai and Theresa, M.K.; Leather Sci. 10 (5) 222 (1963).

15. Rao, V.S., Sudraj Reddy, K.K., Sastry, K.N.S. and Nayudamma Y.; Leather Sci., 15 (7), 189-93 (1968).

16. Menard, E.L.; Mueller, J.M., Thomas, A.F.; Bhatnagar, S.S. and Dastoor, N.J.; Helv. Chem. Acta, 46 ,1801-11 (1963).

17. Matthias, P. Maslinger, E., Zbiral, E.; Monatsh. Chem. 100 (5) 1608-12 (1968).

18. Tschesche, R., Wilhelm, H., Fehlhaber, H.W.; Tetrahedron Lett., 26, 2609-12 (1972).

19. Srivastava, S.K, Srivastava, S.D.; Phytochem., 18 (10) , 1758-9 (1979). (Eng.).

20. Tomoda, M., Ashura, H., Hida, A.; Sayakugaku Zasshi, 23 (2), 45-48, (1969).

21. Okamura, Nobuyuki; Nohara, Toshihiro; Yogi, Akira; Nishioka, Itsuo; Chem. Pharm. Bull 29 (3), 676-83 (1981),(Eng.).

22. Handa, P.C., Dutta, B.K., Jodha, M.R.; Sci., Cult., 36 (5), 286-88 (1970).

23. Sinko, J. And Zellner, J.; Monatsh, 64 , 12-16.(1934).

24. Woo, Woo, Sick; Kang, Sam Sik, Shin, Sang Hyuck, Hanger, Hildebert, Chari, Vedantha Mohan Seligmann, Otto, Wagner, Gunther; Soul Taehak'kyo Saengyak Yonguso Opyukjip, 18 , 17-19, (1979) (Eng.).

25. Woo, Woo, Sick; Kang, Sam Sik, Shin, Sang Hyuck; Hanger, Hildebert, Chari, V.Mohan; Seligmann, Otto;

Ghermaier, Guenther, Phytochemistry (1979).18 (2). 3535 (Eng.).

26. Ikram, M; Ogihara, Y; Yamasaki, K.; J. Nat. Prod. 44 (1). 91-3 (1981) (Eng.).

27. Taran, E.N., Farmatsiya. 4, No.11/12, 20-23 (1941).

28. Tang, Tang-Han and Chao, Yuan-Hsiang; J. Chinese Chem. Soc. 4 . 278-86 (1936).

29. Kawaguti, and Kim, K.W.; J. Pharm. Soc., 60 . 595-6, Abstract 236-36 (1940). (in Eng.).

30. Majumdar, S.; Sarkar, S.M and Dutta, P.C.; J. Ind. Chem. Soc. 33 . 351-52 (1956).

31. Akhmadov., UA . and Khalmatov., Kh.Kh.; Formatsiya. 16 (3). 34-35.(1967).

32. Chughtoi, M.I. D., Khokhar, Irshad, Faseelot, Tahira.; Pak. J. Sci., 30 . (1-6). 136-44. (1978).

33. Akhmadov, U.A. and Khalmatov, Kh.Kh; Polz, Dikorastuschie Rast. Uzb. Ikh. Tspol'zedi "Fan". Uzb. SSR. 154-8.(1968).

34. Akhmadov, U.A. and Khalmatov. Kh.Kh.; Rast Resur; 5 (4). 579-81 (1969).

35. Mehta, T.N.; Rao, C.V.N. and Laxmikantam, V.; India Soap. J. 12 . 44-45 (1953).

36. Blouch, A.K., Hajjatullah, S.; Sci., Res.; 6. (1-2).1-6. (1969).

37. Shibata, S. Negi, Y., Tanaka, O., Doi, O. Phyto chemistry. 9 .(3) 677 (1970).

38. Inoue, O, Ognara, Y. Yamaki, K.J. Chem. Res.(S). 4 . 144; 144-8 (1978).

39. Shanmugavelu, K.G. Rangaswami, G.; J. Sci., Cult., 35 (10). 581-82 (1969).

40. Ahmad, Moghis. V., Husain, S.K., Ansari, A.A., Osman, S.M.; J. Oil. Technol. Assoc. India 11 (3). 70-2. (1979).(Eng.).

41. Tschesche, Rudolf, Shah, Arif H.; Eichardt.; Phytochemistry, 18 (4), 702-4. (1979). (Eng.).

42. Airan, J.N., Rajopdhya, S.B.; J. Indian Chem. Soc., Ind. and News Ed., 12 , 152-54 (1949).

43. Hirst, E.L. and Jones, J.K. N.; J. Chem. Soc., 1659.(1949).

44. Parik, V.M., Ingle, T.R. and Bhide, S.V.; J. Ind. Chem. Soc., 15, 125 (1938).

45. Purdie, T. and Irvine, J.C., J. Chem. Soc., 83 ,1021(1903).

46. Gill, R.E., Hirst, E.L. and Jones, J.K.N., J. Chem. Soc., 1025 (1946).

47. Hampton, H.A., Haworth, W.N. and Hirst, E.L., J. Chem. Soc., 17 9 (1929).

48. Haworth, W.N. Raistrick, H., and Stacey, M., Biochem., J., 29 , 2668 (1935).

49. (a) Oldham, Mary A., and Honeyman, J., J. Chem. Soc., 98 6 (1946).

(b) Hirst, E.L. and Jones, J.K.N. J. Chem. Soc.,1221 (1947)

50. Hirst, E.L. Hough, L. and Jones, J.K.N.; J. Chem. Soc., 928 (1949).

51. Cerezo, A.S.; J. Org. Chem., 30 , 924 (1965).

52. Montgomary R., Smith, F. and Srivastava, H.C.; J. Am. Chem. Soc., 79, 698 (1957).

53. Mikes, O.; 'Laboratory Hand-book of Chromatographic Methods', Ist Ed.(Eng.).; Van Nostrand, P.71 (1966).

54. Rizvi, S.A.I.; D.Phil. Thesis, University of Allahabad, India (1968).

55. Andrews, P.; Hough, L. and Jones, J.K.N.; J. Am. Chem. Soc; 74 , 4029 (1952).

56. Hamilton, J.K., Parlow, E.V. and Thomson, N.S.; J. Am. Chem. Soc.; 82 , 451, (1960).

57. Aspinall, G.O., Rashbrook, R.B. and Kessler, G., J. Chem. Soc., 215 (1958).

58. Meier, H.; Acta Chem. Scand., 14 , 749 (1960).

59. Andrew, P. Hough, L. and Jones, J.K.N.; J. Chem. Soc., 2744 (1952).

60. Hirst, E.L. and Jones, J.K.N.; Dicuss. Faraday. Soc., 7 , 268 (1949).

61. McFarren, E.F; Kathleen Brand and Ratkowski, H.R., Anal. Chem., 23 , 1146 (1951).

62. (a) Tewari, S.N.; J. Anal. Chem., 176 , 604 (1960).
(b) Wilson, C.M.; Anal. Chem., 31 , 1199 (1959).

63. (a) Marier, J.R., Boulet, M.; J Dairy Sci., 42, 1390(1959)
(b) Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.; Anal. Chem., 28 , 350, (1956).

64. Schryver, S.B. Proc. Roy. Soc.(London) B, 82.226 (1910).

65. Whistler, R.L., and Conrad, H.E., J. Amer. Chem. Soc., 76, 1673 (1954).

66. (a) Lederer, E. and Lederer, M.; " Chromatography", Elsvier's P. 166, (1955).
(b) Mikes, O.; "Laboratory Hand-book of Chromatographic Methods , Ist Ed. (Eng.). P. 88 (1966).

67. Trevelyan, W.E.; Proctor, D.P. and Harrison, J.S.; Nature, 166 , 444, (1950).

68. Belcher, R., Fildes, J.E. and Nutten, A.J.; Analyt. Chem. Acta, 13 , 16, (1955).

69. Belcher, R. and Godbert, A.L.; 'Semi-micro Quantitative Organic Analysis', 2nd Ed., P. 164, (1954).

70. Barker, S.A., Foster, A.M., Siddiqui, I.R. and Stacey, M.; Talanta, 1 , 216 (1958).

71. Partridge, S.; Biochem. J., 42 , 238 (1948).

72. Mester, L.; 'Methods in carbohydrate chemistry' ; Editor Royl, L. Whistler, Academic Press, Inc., Vol.II,P.117, (1963).

73. Misaki, A. and Smith, F.; Agr. Food. Chem., 10 ,104.(1962).

74. Mukherjees and Srivastava, H.C. J. Am. Chem. Soc., 77 , 422, (1955).

75. Stephen, A.M., J. Chem. Soc., 4487, (1956).

76. Pastuska, G., J. Anal. Chem., 179, 427 (1961).

77. Smith, F. and Montgomery, R.; ' The Chemistry of plant Gums and Mucilages' , American Chemical Society Monograph Series, Reinhold Publishing Corporation, New York, P. 134 (1959).

78. Souvang, H.O., Kieseling, H., Lindberg, B. and McKay, J.E.; Acta Chem. Scand., 16 , 615, (1962).

79. 'Chromatographic Analysis' General Discussion, Faraday Soc., 7, (1949).

80. Garegg, P.J. and Lindberg, B., Acta. Chem. scand., 14 , 871, (1960).

81. Percival, E.G.V. and Willox, I.C.; J. Chem. Soci., 1603 (1949).

82. Robertson, G.J., Speedie, T.H., J. Chem.Soc., 826.(1934).

83. Chanda, S.K., Hirst, E.L., Jones, J.K.N., Percival, E.G.V., J. Chem. Soc., 1289 (1950).

84. Hampton, H.A., Haworth, W.H. and Hirst, E.L.; J. Chem.,Soc. 1739 (1929).

85. Ethrenthal, E., Rafique, M.C., and Smith, F., J.Chem. Soc., 74 . 1341 (1952).

86. Hirst, E.L., Percival, E.G.V. and Wylam, C.B.; J.Chem. Soc., 189, (1954).

87. White, E.V. and Rao, P.S.; J. Am Chem. Soc., 75, 2617(1953).

88. Brown, F., Halsall, T.G., Hirst, E.L. and Jones, J.K.N.; J. Chem. Soc., 28 (1948)

89. Irvine, J.C. McNicoll, D., Ibid., 97 , 1449. (1910).

90. Cifonelli, J.A. and Smith, F.; Anal. Chem., 26 , 1132. (1954); Ibid., 77 , 1984 (1935).

91. Cyong, Jyong-chyul; Ham Abusa, Kiyomichicoviant, Med. Res. cent. Kitasato Inst. Tokyo, Japan, 108, Phytochemistry, 19 (12), 2747-8 (1980). (Eng.)

92. Hough, L. and Powell, D.B.; J. Chem. Soc., 16 (1960).

93. Whistler, R.L., Tu, C.C.; J. Amer. Chem. Soc. 74 , 3609, (1952).

94. Curtis, E.J.C., Jones, J.K.N.; Cand J. Chem., 38 ,1305, (1960).

95. Haward B.H.; Biochemical J., 67 , 643 (1957).

96. Srivastava, H.C. and Smith, F. J. Am. Chem. Soc.,72 , 982, (1957)

97. Whistler, R.L., Bachrach, J. and Tu, Chen-chuan; J. Am. Chem. Soc., 74, 3059 (1952).

98. Whistler, R.L., Tu, C.C., J. Am. Chem. Soc., 73, 1389.(1951).

CHAPTER - III

A WATER SOLUBLE NEUTRAL POLYSACCHARIDE FROM THE UNRIPE FRUITS OF MUSA SAPIENTUM LINN.

III.1. The present Chapter describes the isolation and structural elucidation of a water soluble neutral polysaccharide from the unripe fruits of Musa sapientum Linn. belongs to the family Musaceae[1].

The plant Musa sapientum Linn., is commonly known as Kela (Banana). A tropical fruit, having its origin in the Malayan penunsula, the 'Banana' an African name, is one of the cheapest and most liked nutritive fruit. This is one of the most popular and widely grown plant of the tropics and subtropics, produced in enormous quantities for export. The plant is considered symbolic of prosperity. There are numerous varieties of Banana differing in size, shape, colour, flavour of fruit and suitability for handing and export.

In India, all Banana called Plantain[2] but the word is usually used for the large fruited, starchy or cooking variety important for human food in many countries especially tropical Africa, these belongs to the species Musa paradisiaca while most eating varieties belongs to species Musa sapientum.

The fruit is three to eight inches (7.5 to 20 cms) in length with a thickness of 1 to 1.5 inches (2.5 to 3.5 cms). Some varieties have a thick skin while others a thin skin. The skin may be yellow or red. The fruit has a whitish sweet pulp with agreeable flavour. The plant is indigenous in Bihar and the Eastern Himalayas upto 4000 ft. cultivated throughout India[1].

Bananas are a good source of carbohydrates, minerals and vitamins with a high calorific value. Demulcent and astringent properties are attributed to them. Popularly used in dietetic treatment of sprue, chronic dysentries and diarrhoeas. Ripe fruit is Peels of green bananas are useful as dermatol therapeutic agent against e.g. eczema, skin eruption, chaps or burn and as skin cream against wrinkle[106]. Unripe fruit is astringent and ripe fruit is anticorbutic used as a mild demulcent, astringent, diet in cases of dysentery[1].

Banana fruit is mild laxative. It aids in combating diarrhoea and dysentery and promotes the healing of intestinal lesions in ulcerative colitis. Ripe fruit is also useful in diabetes, uremia, nephritis, gout, hypertension and cardiac diseases. The stalk of the fruited plant[1] is given to pigs in China for kidney worms. The ash of the root or of the whole plant is anthelmintic[1].

The work done in the past years on this genus was surveyed and the details of it are given in the tabular forms

	Genus	Species	Constituents	Parts	Reference
1.	Banana	-	Determination of Ascorbic acid (Vitamin C)	-	(1936)[3]
2.	Banana	-	Riboflavin content (Vitamin B_2)	-	(1941)[4]
3.	Banana	-	Vitamin A, C and Vitamin B-Complex	-	(1946)[5]
4.	Banana	-	Xanthophylls, Kryto-xanthin, lycopene, neolycopene, neo-β-carotene-U, β-carotene, neo-β-carotene-B, α-carotene (carotenoid pigments).	-	(1949)[6]
5.	Banana	-	Composition of Ascor-bic acid content	-	(1950)[7]
6.	Banana (Taiwan)	-	Separation & charact-erization of fructosyl sucrose	-	(1963)[8]
7.	Banana (Taiwan)	-	Partial hydrolysis of fructosyl sucrose with β-D-fructofurnoside yielded fructose, glu-cose, sucrose & a redu-cing disaccharide (on further hydrolysis yielded glucose & sucrose) Enzymatic hydrolysis yielded monosaccharide residues	-	(1964)[9]
8.	Banana	-	Biochemistry, Physiology & Nutritive value of Banana. Analysis of	-	(1968)[10]

Genus	Species	Constituents	Parts	Reference
		amino acid, carbohydrate mineral, tannin, organic acid, Proteins & fats, vitamins (except for B_2) starch content (20-32%).		
9. Banana	-	Cycloeucalenol, cycloartenol & 24-methylene cycloartanol, esters of palmatic acid, stigmasterol, campesterol, & β- sitosterol	Rhizome, stalk and leaves	(1969)[11]
10. Banana	-	Rutin, quarcetinglycoside caffeic acid, catechin, ferulic acid, chlorogenic acid, p-coumaryl quinic acid, cinnamic acid and derivatives, dopa, novadrenaline, dopamine, leucoanthocynidin, 5-hydroxy tryptophan, L-tryptophan, 5-hydroxy tryptamine & trytamine.	-	(1970)[12]
11. Banana	-	Physiochemical nature of Banana pseudostem starch (Amylase content)	-	(1970)[13]
12. Banana (Taiwan)	-	Determination of carbohydrate (Galactose, galactouronic acid, glucose, arabinose, fructose and Rhamnose in polysaccharide), starch 1.85%, soluble starch & dextrin 2.22 %, and sucrose 9.28 %, glucose 4.69% and fructose 1.16%	Skin and skin interiors and pulp	(1969)[14]

Genus	Species	Constituents	Parts	Reference
13. Banana	-	Pelagonidin, Cyanidin, Peonidin, delphinidin, Petunidin, malvidin, (Anthocyanins)	Bracts	(1954)[15]
14. Banana	-	Melanins	Buds and flowers	(1967)[16]
15. Banana	-	Glutamic acid	Skin of Banana	(1959)[17]
16. Banana	-	Glucose, fructose, sucrose from the alcoholic extract of Banana	Green, ripe and perisarp of fruit	(1964)[18]
17. Banana	-	Dopamine	Fruits	(1964)[19]
18. Banana	-	Sucrose, glucose and fructose	Edible part of fruits	(1967)[20]
19. Banana	-	Constitution of Banana starch(on hydrolysis affords 97% glucose	Unripe fruits	(1940)[21]
20. Banana	-	Extraction, purification and amylase content of some starches, Amylase content of green banana contain 25%	Green Banana	(1971)[22]
21. Banana	-	5-hydroxy tryptamine, phenyl Butazone.	Unripe banana	(1964)[23]
22. Banana (Columbian banana)	-	Chemical & Biochemical characterisation of Banana. It is a source of starch, Protein, minerals(K, Mg, Ca, Fe, Mn, Na) tannin, pectins, disaccharide, alkaloids, & fiber content.	Ripe & green peel	(1970)[24]

Genus	Species	Constituents	Parts	Reference
23. Banana	-	Long chain free fatty aldehyde	Fruits lipid	(1979)[25]
24. Banana	-	Maltose, glucose, sucrose, fructose & 3 or 4 other sugars.	Green & ripe pulp	(1955)[26]
25. Banana	-	Citric acid, L-Malic acid, (Organic acids)	Green & ripe Banana	(1954)[27]
26. Banana	-	Dopa, Dopamine, serotonin, Indole-3-acetic acid & norepinephrine	Peel	(1968)[28]
27. Banana (2-Varieties of Havana)	-	Vitamin B_1	Ripe	(1943)[29]
28. Banana	-	Vitamin A, B and C content.	Ripe	(1937)[30]
29. Banana	-	Lipid, 18 fatty acids C-C chain between 6-22, unsaturated fatty acid of 16-18C	Ripe peel & pulp	(1969)[31]
30. Banana	-	Alkanoic acid, C nos (C_6-C_{22}) 4-unsaturated acid, palmatic acid	Pulp and pericarp of fruits	(1964)[32]
31. Banana	-	Noradrenaline, serotonin, 3-(3,4,-dihydroxy phenyl) amine (Phenantolamine)	Pulp and stalk juice	(1962, 1963)[33]
32. Banana	-	An antacid, demulcent, & prednisolone.	Pulp	(1965)[34]
33. Musa	Sapientum	Antibacterial substances (Antibiotic)	Leaves	(1949)[35]

Genus	Species	Constituents	Parts	Reference
34. Musa	Sapientum	$C_{29}H_{50}$(3-β-OH-Δ^5-sterol) & several Derivs.of AC,-di Br,-di-H,and n-C_{29}-H_{60}	Leaf	(1955)[36]
35. Musa	Sapientum	n-alkanes from C_{19}-30, several substituted nuphthales,phenanthreanes & related aromatics.(Aromatic hydrocarbon).	Leaf	(1966)[37]
36. Musa	Sapientum	Hypoglycemic substances	Flowers	(1966)[38]
37. Musa	Sapientum	Triterpene ketone (31-norcycloleudanone)	Peel	(1970)[39]
38. Musa	Sapientum	Cycloartenyl palmitate (cholesteric esters)	Peel	(1970)[40]
39. Musa	Acuminata	A new Leucoanthocyanidin	Seed	(1962)[41]
40. Musa	Acuminata	Proanthocyanidin glycoside	Seeds	(1971)[42]
41. Musa	Cavendishii	Analysis of oligosaccharide by the examination of paper chromatography of 80% aq.EtOH Extract.	-	(1964)[43]
42. Musa	Cavendishii	Sucrose,glucose, fructose,maltose, xylose,Raffinose, Rhamnose,mannose	Rhizome and stalk	(1965)[44]
43. Musa	Cavendishii dwarf (2 varieties of China)	Malic, citric,phosphoric acids	pulp	(1963)[45]

Genus	Species	Constituents	Parts	Reference
44. Musa	Paradisiaca (Portorico plantain)	Vitamin A & B	-	(1927)[46]
45. Musa	Paradisiaca	Vitamin B(B_2) or G	-	(1930)[47]
46. Musa	Paradisiaca	D-glucitol (Sorbitol)	Leaves	(1966)[48]
47. Musa	Paradisiaca	Extraction of pectins & mucilage content.	Seeds	(1929)[49]
48. Musa	-	Catecholamine & serotinin(Monoamine)	Various organs & tissue	(1968)[50]
49. Musa	(Psyllium)	Pharmacognosy	-	(1934)[51]

Different parts of this genus have been investigated from different plant products as have already been described in literature, but no neutral polysaccharide has been mentioned on the unripe fruits of Musa sapientum Linn. uptill now.

Therefore an attempt has been made for isolation and structural elucidation of the polysaccharide from the unripe fruit of this important plant Musa sapientum.

III.2 STRUCTURAL ELUCIDATION OF NEUTRAL WATER SOLUBLE POLYSACCHARIDE FROM THE UNRIPE FRUITS OF MUSA SAPIENTUM

III.2.1 RESULTS AND DISCUSSION

A new water soluble polysaccharide has been isolated

from the defatted unripe fruits of M.sapientum , by extracting with 1% acetic acid and precipitating with excess of ethanol. The polysaccharide was repeatedly purified till the ash content reduced in minimum. The homogeneity of the polysaccharide checked by :

(i) Fractional precipitation.

(ii) Zone - electrophoresis.

(iii) Acetylation and deacetylation.

The polysaccharide was dissolved in water and separated into three fractions by fractional precipitation with different volume of ethanol. All the three samples were analysed quantitatively by the method of Hirst and Jones[54]. The results were essentially identical to the original polysaccharide indicating the polysaccharide to be homogenous.

The portion of the polysaccharide was separated by Zone - electrophoresis method in borate buffer (pH 9.3). After completion of the experiment, a plot of the absorbance against segment numbers showed only a single sharp peak indicating the polysaccharide to be homogeneous.

The homogeneous polysaccharide was acetylated with acetic anhydride and sodium acetate. The acetylated product showed optical rotation $[\alpha]_D^{25}$ + 29.5° (in chloroform,C. 0.85%). On deacetylation, it gave a polysaccharide having the same optical activity as the original one. Thus it confirmed the homogeneity of the polysaccharide.

III.2.2 The polysaccharide was slowly soluble in water.

$[\alpha]_D^{25}$ +73.6° (in water, c, 0.8%), ash content 0.75% .
The polysaccharide was found to be free of nitrogen, sulphur, and halogens. The methoxyl, uronide and acetyl percentage were found to be negligible.

III.3 The complete acid hydrolysis of the polysaccharide with 2N sulphuric acid followed by paper chromatographic analysis of the hydrolysate revealed the presence of three sugars, D-galactose, D-mannose and D-xylose. The identity of the sugars was confirmed by their specific optical rotations, preparation of their crystalline derivatives and co-chromatography with authentic samples.

The quantitative estimation of the mono-saccharide components by periodate oxidation taking Ribose as a reference sugar showed that mannose, galactose and xylose are present in the molar ratio 4:1:1 in the polysaccharide.

The graded hydrolysis of the polysaccharide with 0.05N sulphuric acid and subsequent paper chromatographic analysis of the hydrolysate taking out at various intervals, revealed that galactose was liberated first followed by the liberation of xylose and mannose respectively. This shows that mannose units are linked together forming the backbone (main chain) of the polysaccharide and galactose and most of the xylose units are linked as terminal groups. The easy liberation of galactose units indicate that most probably they are linked to the main chain at peripheri through -linkages.

III.4 The polysaccharide was methylated first by Haworth's

method using dimethyl sulphate and alkali[55] followed by Purdie's method[56] giving a methylated polysaccharide having optical activity $[\alpha]_D^{25}$ + 40° (in chloroform,C,1.5%) -OCH_3 . 0. 44.06% . The complete hydrolysis of the methylated polysaccharide and paper chromatographic analysis of the hydrolysate in solvent (A), revealed the presence of five methylated sugars. The methylated sugars were separated on a preparative scale by chromatography on Whatman No.3 filter paper. The following methylated sugars were identified.

(1) 2,3,4,6-tetra-O-methyl-D-galactose;

(2) 2,3,4 -tri-O-methyl-D-xylose;

(3) 2-O-methyl-D-xylose;

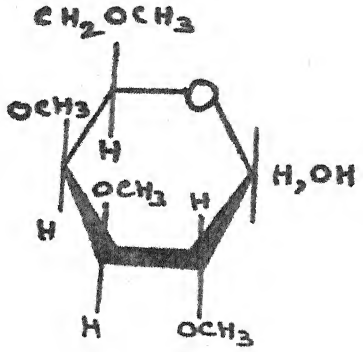
(4) 2,3-di-O-methyl-D-Mannose;

(5) 2,3,6-tri-O-methyl-D-mannose.

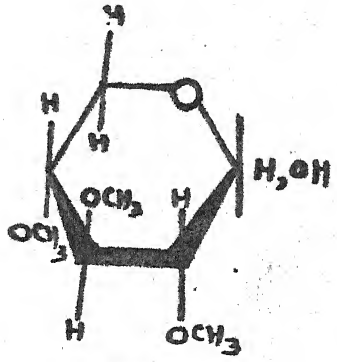
Methylated sugar (1), had R_{TMG} in solvent(A), 0.89, m.p. 71-73° $[\alpha]_D^{25}$ +123° (in water,C, 0.5%). On treatment with ethanolic aniline gave 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine, m.p. 188-89°, $[\alpha]_D^{25}$-80° (in acetone, C,1.1 %). Therefore, the identify of the methylated sugar 1, is established as 2,3,4,6-tetra-O-methyl-D-galactose.

Methylated sugar (2), was obtained as a syrup could not be recrystallised, R_{TMG} in solvent (A), 0.95, $[\alpha]_D^{18}$ + 19.2° (in water,C, 0.38). On treatment with ethanolic aniline it gave, 2,3,4-tri-O-methyl-D-xylopyranosyl anilide, m.p. 95-96°, $[\alpha]_D^{22}$- 80° (in ethanol,C, 2.5 %). The sugar in this fraction was thus identified as 2,3,4-tri-O-methyl-D-xylose.

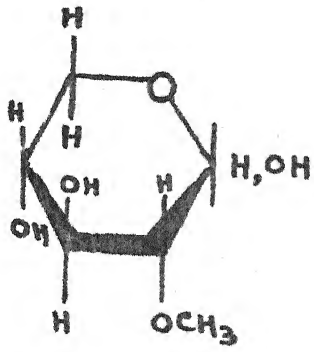
Methylated sugar (3), had R_{TMG} value in solvent(A),


CH2OCH3
OCH3
H
H
OCH3
H
H,OH
H
OCH3

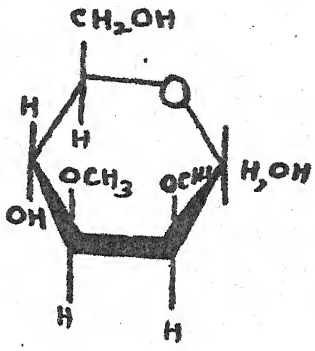
I


H
H
H
H,OH
OCH3
H
OCH3
H
OCH3

II


H
H
H
H,OH
OH
H
OH
H
OCH3

III


CH2OH
H
H
H,OH
OCH3
OCH3
OH
H
H

IV


CH2OCH3
H
H
H,OH
OCH3
OCH3
OH
H
H

V

0.39, $[\alpha]_D^{25}$ -25° (in water,C, 2.3%), m.p. 132-33°. It formed 2,O-methyl-D-xylose anilide, m.p. 122-24° $[\alpha]_D^{25}$+210° (in ethylacetate,C,0.9%). Its diacetate, 2,O-methyl, 3,4-diacetate had m.p., 76-77°, $[\alpha]_D^{25}$- 39° (in chloroform,C, 3.0%). Thus the above observations confirmed that the methylated sugars, I is 2-O-methyl-D-xylose.

Methylated sugar (4), was also obtained as a syrup, R_{TMG} in solvent (A) 0.154, $[\alpha]_D^{26}$- 16.6°(C, 1.8 % in water). It formed 1,4,6, p-nitrobenzoate with p-nitrobenzoyl chloride, m.p. 190-92° $[\alpha]_D^{26}$+ 63° (in chloroform C,1.5%) which shows that the methylated sugar (4) is 2,3-di-O-methyl-D-Mannose.

Methylated sugar (5), R_{TMG} in solvent(A), 0.82, $[\alpha]_D^{25}$-12.5° (in water, C, 1.6 %) formed 1,4-bis-p-nitrobenzoate, m.p. 186-87° $[\alpha]_D^{26}$ +32° (in chloroform,C, 0.5 %). On oxidation with bromine water, it gave a lactone, which on treatment with phenyl-hydrazine formed 2,3,6-tri-O-methyl-D-Mannonic acid phenyl hydrazide, m.p. 128-30°. This indicates that the methylated sugar (5), is 2,3,6-tri-O-methyl-D-Mannose.

The quantitative estimation of methylated sugars by the method of Hirst and Jones[57] showed that sugars 1,2,3,4,5 were present in the molecular ratio, 2:1:1:2:6.

The studies indicate that galactose units in the polysaccharide occupy terminal positions as nonreducing end groups from which 2,3,4,6-tetra-O-methyl-D-galactose (1), arises on hydrolysis of the methylated polysaccharide. A

large portion of (5), 2,3,6-tri-O-methyl-D-mannose(6 moles) indicates that the backbone of the polysaccharide consists of mannose units linked through 1→4 linkages. Detection of 2,3,-di-O-methyl-D-mannose (2 moles) shows that two mannose units in the main chain per repeating unit of the polysaccharide are linked at position 6 in addition to -1 and -4 - positions. Isolation of 2-O-methyl-D-xylose(1mole) made an idea that one mole of xylose unit per repeating unit of the polysaccharide is linked at position 1,3,4. Presence of 2,3,4-tri-O-methyl-D-xylose (1 mole) shows that one xylose unit in the polysaccharide occupy terminal position through 1→3 linkage.

III.5 Determination of terminal groups by periodate oxidation and subsequent titration of liberated formic acid, corresponds to 0.782 moles of formic acid per 100 g of the polysaccharide, is supposed to consist of 12 sugar moieties of which 2 units of galactose and one unit of xylose form terminal groups. Considering such a repeating unit, the terminal groups were found 24.91% as determined by periodate oxidation studies which is identical to that revealed by methylation studies (25.03%).

III.6 The partial acid hydrolysis of the polysaccharide followed by paper chromatographic separation on preparative scale afforded five (5) oligosaccharide. The following oligosaccharide were detected :

1. Mannotriose, O-β-D-mannopyranosyl(1→4)-O-β-D-mannopyranosyl (1→4)-D-mannopyranose.
2. Spinelibiose, 6-O-α-D-galactopyranosyl-D-mannopyranose.

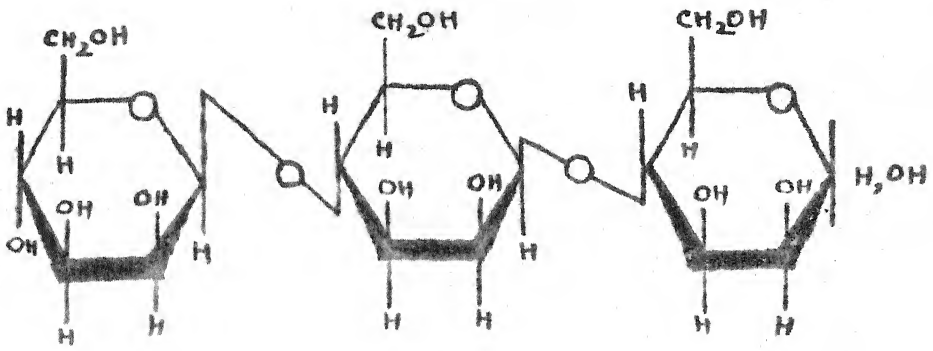


Fig - 1

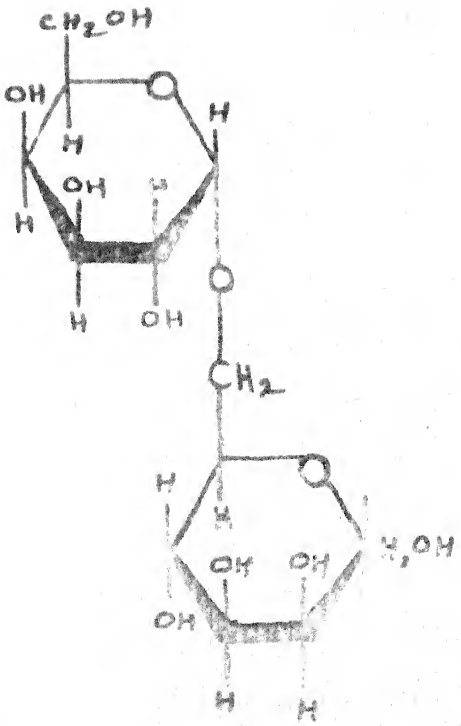


Fig - 2

3. 6^2-α-galactosyl mannobiose, O,α-D-galacto-pyranosyl (1→6)-O-β-D-mannopyranosyl-(1→4)-D-Mannopyranose.

4. Mannobiose, 4-O-β-D-mannopyranosyl-D-mannopyranose.

5. Rhodymenabiose, (O-β-D-xylopyranosyl(1→3)-O-β-D-xylopyranose).

Oligosaccharide (1), m.p. 168-69°, $[\alpha]_D^{30}$-18.8°(in water,C, 1.8%) was chromatographically pure in solvent (C), (G) and (F). It was shown to be monohydrate of trisaccharide on the basis of its equivalant weight, 264.8. Acid hydrolysis of the oligosaccharide yielded only mannose. Partial acid hydrolysis yielded mannose and mannobiose which were identified by co-chromatography with the authentic samples. The identity was also confirmed by the periodate oxidation data which showed the liberation of 2.10 moles of formic acid with the consumption of 5.3 moles of meta periodate per moles of sugar. Hence the oligosaccharide was identified to be -O-β-D-manno pyranosyl-(1→4)- O- β -D-manno pyranosyl-D-mannopyranose.(Fig. 1).

Oligosaccharide (2), was isolated in crystalline form having the physical constants identical with those reported for 6-O-α-D-galactopyranosyl-D-mannopyranose. It reduced Fehling solution and Tollen's reagent having m.p. 200-02°, $[\alpha]_D^{32}$+ 120.5° (in water, C,0.45 %) and was found to be a single entity by paper chromatography in three different solvent systems (A), (B) and (C). The paper

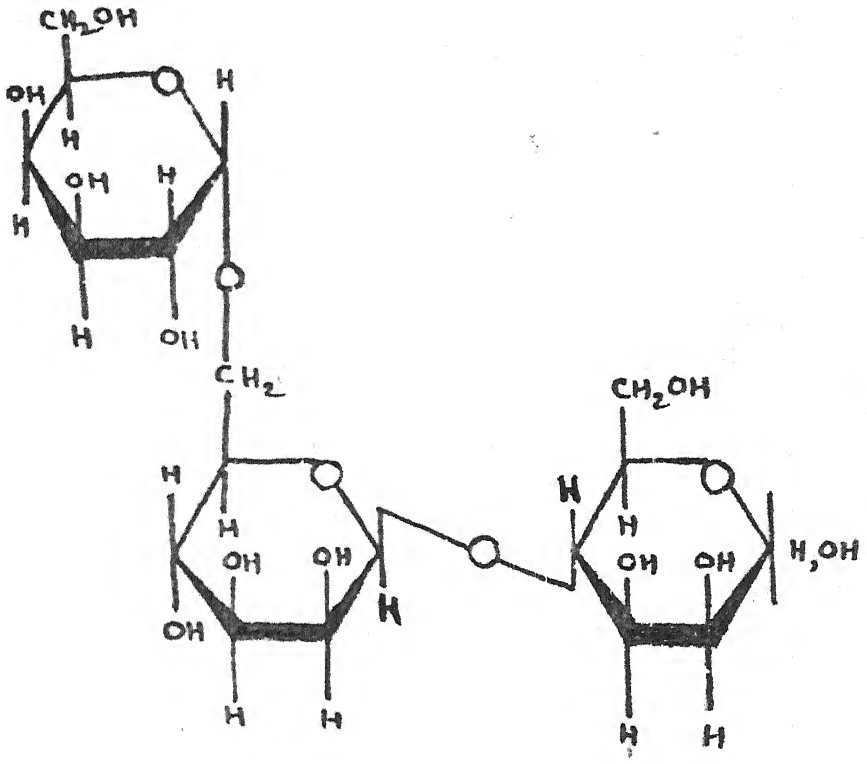


Fig-3

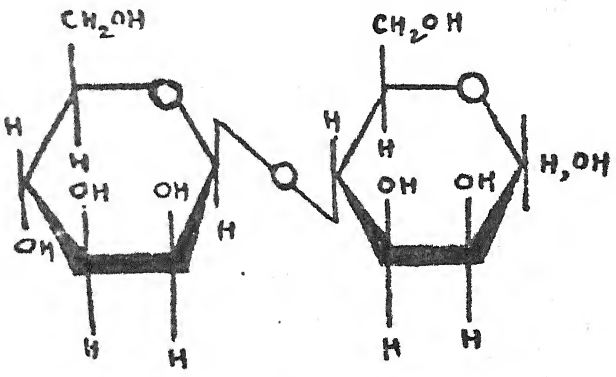


Fig-4

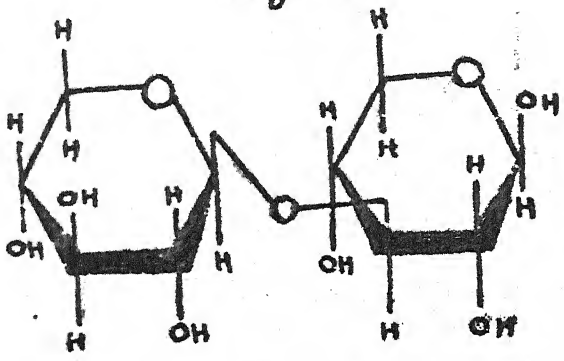


Fig-5

chromatographic analysis of the completely hydrolysed sugar revealed the presence of galactose and mannose. The quantitative estimation by the method of Hirst and Jones[54] showed the molar ratio 1 : 1 between the two sugars in the oligosaccharide. The equivalent weight, 174.2, showed it to be a disaccharide. The periodate oxidation studies afforded the liberation of 3.2 moles of formic acid and consumption of 5.24 moles of periodate per mole of disaccharide. The liberation of 3.2 moles of formic acid from the disaccharide indicates that there is 1→6 linkage between galactose and mannose units. As the disaccharide could not be hydrolysed with emulsin, it is inferred that galactose and mannose have ∝-linkage between them. On the basis of above evidences, the oligosaccharide was identified to be epimelibiose, 6-O-∝-D-galactopyranosyl-D-mannopyranose and identity was further confirmed by co-chromatography with an authentic sample. (Fig. 2).

Oligosaccharide (3), was crystallised from ethanol. m.p. 226-27°. $[\alpha]_D^{32}$ + 98.5° (in water, C, 0.5%). It was shown to be a single entity by paper chromatography in solvent (G), (C) and (S). (Page 94). It reduced Fehling solution and Tollen's reagent. The complete acid hydrolysis of sugar and subsequent paper chromatographic examination revealed the presence of galactose and mannose. The quantitative estimation by the method of Hirst and Jones[54] showed that galactose and mannose are present in the oligosaccharide in the ratio 1:2. The equivalent weight, 262.8, showed it to be a trisaccharide. The periodate oxidation studies showed the liberation of 3.18 moles of

formic acid with the consumption of 6.30 moles of meta-periodate. Partial acid hydrolysis followed by paper chromatographic examination showed the presence of manne-biose and epimaltbiose besides galactose and mannose. Their identity were confirmed by co-chromatography with authentic samples. The oligosaccharide was, thus identified as O-α-D-galactopyranosyl (1→6)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose. (Fig. 3).

Oligosaccharide (4), was also isolated in crystalline form, having the physical constants identical with those reported for 4-O-β-D-mannopyranosyl-D-mannopyranose. It reduced Fehling solution and Tollen's reagent. The sugar on acid hydrolysis yielded only mannose while the equivalent weight of the sugar, 174.8, corresponds to a hexose disaccharide. Enzymatic hydrolysis with emulsin showed the presence of β-linkage between the two mannose units. The periodate oxidation showed the liberation of 2.14 moles of formic acid with the consumption of 4.22 moles of meta-periodate per mole of the sugar. Hence the oligosaccharide was assigned the structure, 4-O-β-D-mannopyranosyl-D-mannopyranose. The identity was confirmed by co-chromato-graphy with an authentic sample. (Fig. 4).

Oligosaccharide (5), a crystalline sugar, m.p. 191°, $[\alpha]_D^{22}$ - 21° (in water, C, 2.92 %), was found to chromato-graphically pure in the solvents(F)and(a). The sugar on acid hydrolysis yielded only xylose while the molecular weight of the sugar 296 corresponded to a pentose disaccharide. Enzymic hydrolysis with emulsin showed the

presence of β-linkage between two xylose units. The periodate oxidation showed the consumption of 3.24 moles of metaperiodate with the liberation of 1.18 moles of formic acid per mole of the sugar. The identity was confirmed by co-chromatography with an authentic sample. The oligosaccharide is, therefore identified to be O-β-D-xylopyranosyl-(1→3)- O-β-D-xylopyranose. (Rhodymenabiose). (Fig. 5).

XII.7 On the basis of the results obtained so far particularly from the methylation studies, graded and partial acid hydrolysis, the following valuable informations could be derived.

(i) The main chain of the polysaccharide consists of β-(1→4) linked mannose units.

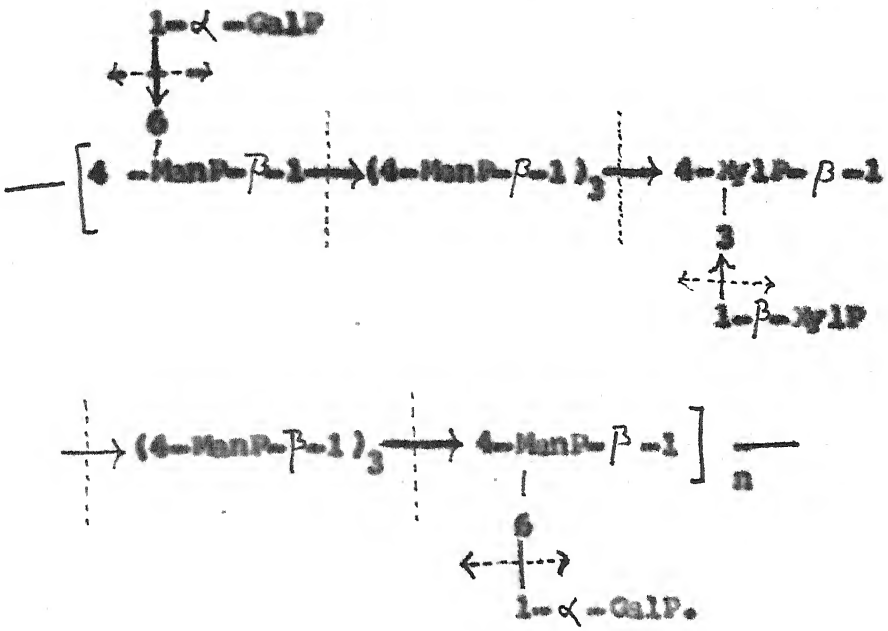
(ii) One xylose unit per repeating unit of the polysaccharide is also linked in the main chain through β-(1→4) linkage.

(iii) Galactose units are linked in side chain to the main chain through α-(1→6) linkages.

(iv) One xylose unit is present in the side chain through β-linkage, position. 1,3, and 4.

(v) β-(1→3) linkage between xylose and xylose units is present in the side chain only.

Taking all the experimental evidences into consideration together with the structures of different oligosaccharide, the following most probable structure has been assigned to the polysaccharide from the fruits of *Musa sapientum*.



GalP = Galactopyranose

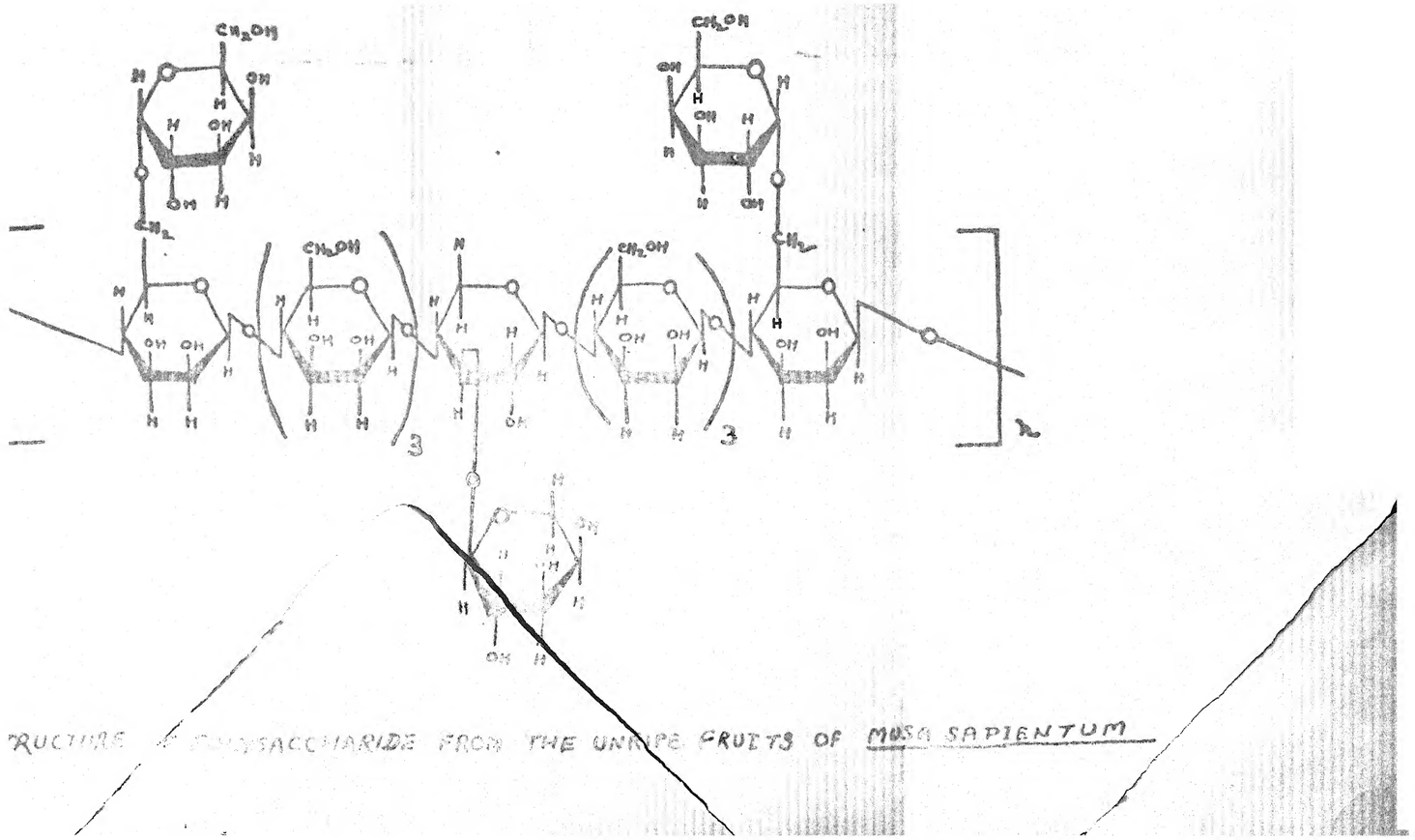
XylP = Xylopyranose

ManP = Mannopyranose.

The above structure containing 12 units of hexose and pentose monosaccharides per repeating unit, which fully explains the formation of oligosaccharides as obtained by partial acid hydrolysis and agrees well with the analytical data of the polysaccharide. The dotted line and doubly arrowed lines show the probable mole of fission of linkages during the partial acid hydrolysis. The arrowed dotted lines indicate secondary hydrolysis.

The polysaccharide such as described above should consume 14 moles of metaperiodate with the liberation of 3 moles of formic acid per repeating unit of 12 sugar units. The actual consumption of periodate and liberation of formic acid have been determined to be 14.15 moles and 2.98 respectively per repeating units of polysaccharide which are in close agreement to the calculated values.

Possibility of the similar structure cannot be completely ruled out but they are less probable because the formation of oligosaccharide as obtained in the present case might not be possible.



STRUCTURE OF POLYSACCHARIDE FROM THE UNRIPE FRUITS OF MUSA SAPIENTUM

III.8 EXPERIMENTAL

Experimental techniques were same as described on (Page 34). Paper chromatography was performed at room temperature by descenting technique on Whatman No.1 filter paper unless stated otherwise using following solvent systems:

(A)	n-Butanol-ethanol-water	(5:1:4)[58]
(B)	n-Butanol-acetic acid-water	(4:1:5)[59]
(C)	n-Butanol-isopropanol-water	(11:1:5)[60]
(D)	Benzene-ethanol-water	(169:47:15)[61]
(E)	Butanone - water	(10 : 1)[62]
(F)	Ethylacetate - pyridine-water	(10:4:3)[63]
(G)	Ethyl acetate-pyridine-water	(2:1:2)[64]
(H)	n-Butanol-ethanol-water	(40:11:19)[65]
(I)	n-Butanol-pyridine-water	(6:4:3)[66]

The spots were located by spraying the chromatogram with aniline hydrogen phthalate[67] and heating it at 120° for 10-15 minutes. Spectrometric determination were carried out by a modification of phenol-sulphuric acid method[68]. Klett-Summerson photoelectric colorimeter was used for measuring the absorbance.

III.9 ISOLATION OF THE POLYSACCHARIDE

The dried and crushed unripe fruits (3 Kgs) were extracted successfully with petroleum ether (60-80°) and ethanol. The polysaccharide extracted from the extracted unripe fruits of *Musa sapientum* by the repeating the process as given on page 35. A colourless fibrous precipitate of the crude polysaccharide was obtained. It was filtered, washed

with absolute ethanol and dried in vacuum at room temperature (49 g. ash content 3.15%).

III.10 PURIFICATION OF THE POLYSACCHARIDE

The dried crude polysaccharide was dissolved in distilled water (2 litres) containing 1% acetic acid with constant stirring. The solution was filtered and was added very slowly to ethanol (8 litres) with constant stirring and kept over-night. The precipitated polysaccharide was filtered and the above process was repeated four times, to get a white fibrous mucilage. (35.8 g. ash 0.75%).

III.11 HOMOGENEITY OF THE POLYSACCHARIDE

The homogeneity of the polysaccharide was checked by the following methods.

III.11 (a) Fractional Precipitation

The pure mucilage (5g) was fractionally precipitated into two fractions (Fraction I and Fraction II). Both the fractions alongwith the original polysaccharide were hydrolysed and quantitatively estimated by the usual method as described on page 44. The ratio of mannose, galactose and xylose in both the fractions was found almost the same(4:1:1) indicating the purified polysaccharide was to be homogeneous.

III.11 (b) Zone - electrophoresis

Polysaccharide (300 mg) was taken for zone-electrophoresis and similar procedure was adopted as described on page 38.

The corrected absorbance reading (Table-1) are as follows, so obtained were plotted against the distance from

the anode, that is segment number which showed only one sharp peak indicating the polysaccharide to be homogeneous.

TABLE - 1

Segment number	Klett reading of elute	Blank Klett reading	Corrected Klett reading	Absorbance
1	26	25	1.0	0.002
2	27	25	2.0	0.004
3	26	24	2.0	0.004
4	28	25	3.0	0.006
5	27	25	2.0	0.004
6	25	23	2.0	0.004
7	25	21	4.0	0.008
8	25	22	3.0	0.006
9	24	22	2.0	0.004
10	24	23	1.0	0.001
11	25	22	3.0	0.006
12	30	25	5.0	0.010
13	28	25	3.0	0.006
14	35	33	2.0	0.004
15	37	21	16.0	0.032
16	50	21	29.0	0.058
17	38	21	17.0	0.034
18	29	26	3.0	0.006
19	29	27	2.0	0.004
20	25	24	1.0	0.002
21	25	21	4.0	0.008
22	25	21	4.0	0.008
23	24	22	2.0	0.004
24	24	21	3.0	0.006
25	28	25	3.0	0.006
26	28	22	6.0	0.012
27	27	25	2.0	0.004
28	23	21	2.0	0.004
29	25	21	4.0	0.008
30	25	22	3.0	0.006

Absorbance was measured on 5 ml portion of coloured solution.

$$\text{Absorbance} = \frac{2 \times \text{Klett reading}}{1000}.$$

III.11 (c) Acetylation and Deacetylation

The pure mucilage (2g) was mixed thoroughly with anhydrous sodium acetate (10 g) and the mixture was suspended in acetic anhydride (30 ml) and further process was repeated as on page 37 . The acetylated polysaccharide (1.5g) was obtained having the optical rotation $[\alpha]_D^{25}$+29.5 (in chloroform, C,0.85%).

The dried acetylated polysaccharide (1.0 g) was dissolved in acetone (30 ml) and 50% potassium hydroxide solution (30 ml) was added to it. The deacetylation was carried in the usual manner[69], as given on page 38 . The deacetylated polysaccharide (0.5g) having $[\alpha]_D^{25}$+ 73.5° (in water,C,0.78 %) with close agreement to original one indicating the homogeneity of the polysaccharide.

III. 12 ASH CONTENT

The dried polysaccharide (0.4g) was ignited in a silica crucible which previously heated to a constant weight. After ignition, the crucible cooled in a desicator and weighed. From the weight of residue (0.0014 g), the ash content was calculated 0.75% .

III. 13 PHYSICAL AND CHEMICAL EXAMINATION

It was a fibrous white powder, very light in weight, slowly soluble in water. $[\alpha]_D^{25}$+ 73.6° (in water, C,0.85%). For the purpose of optical rotation, the solution was filtered through a sintered glass funnel to get a clear solution and the amount of polysaccharide in the solution was determined colorimetrically. The polysaccharide was found to be free

from nitrogen, sulphur and halogens. On treatment with Fehling's solution, it formed an insoluble copper complex but did not reduce it.

III. 14 EXAMINATION OF FREE SUGARS

The polysaccharide was examined for free sugars by applying three spots of its solution in a water on a strip of Whatman No.1 filter paper (15 x45cms) and developed in solvent (A) as described on page 40. The three spray reagents naphtharesorcinol and trichloroacetic acid[70] and aniline hydrogen phthalate[65] and silver nitrate in acetone followed by ethanolic sodium hydroxide[71] on three different strips of above paper showed no spot, hence it showed that the polysaccharide was free of any free sugar.

III. 15 METHOXYL GROUPS DETERMINATION

The percentage of methoxyl groups was determined by the method of Belchar, Fildes and Nutten[72] and was found to be negligible.

III. 16 ACETYL GROUPS DETERMINATION

The method by Belcher and Godbert[73] was followed for the determination of acetyl group percentage with and without mucilage which was found in significant (0.96%).

III. 17 URONIDE CONTENTS DETERMINATION

The uronide contents were found to be negligible by the semi-micro method of Baker, Foster, Siddiqui and Stacey[74].

III. 18 HYDROLYSIS OF POLYSACCHARIDE AND DETERMINATION OF MONOSACCHARIDE

The purified mucilage (1.5 g) was dissolved in 2N

sulphuric acid (100 ml) and was hydrolysed on a water-bath for about 24 hours. The hydrolysed polysaccharide was neutralised with barium carbonate, filtered and concentrated under reduced pressure. The hydrolysed was examined paper chromatographically for monosaccharides.

XII.18 (a) Paper Chromatography

The spots of the hydrolysate were applied on two sheets of Whatman No.1 filter paper. The papers were developed separately in solvents (A) and (B) by descending unidimensional technique. The chromatograms were air-dried and sprayed with aniline hydrogen phthalate. On heating them in an oven at 120° each chromatogram showed three spots. The R_g and R_G values of the three spots corresponded to D-mannose, D-galactose and D-xylose as given in the table -2.

TABLE - 2

Sugar identified	Solvent (A)		Solvent (B)	
	R_G found	R_G given[75]	R_g found	R_g given[59]
D-Mannose	0.12	0.11	0.20	0.21
D-Galactose	0.06	0.07	0.17	0.16
D-Xylose	0.14	0.15	0.27	0.28

G = 2,3,4,6-tetra-O-methyl-D-glucose.

The identity of the three sugars was further confirmed by co-chromatography with authentic sample of the sugars in the same solvent systems.

XII.18 (b) Column Chromatography

A portion of hydrolysate was dissolved in a small

amount of aqueous methgnol(1:1) and absorbed over a column of cellulose (2x 35 cms). The column was left over-night and the separation was effected with solvent (A). Fractions amounting to 10 ml were collected and checked by paper chromatography with authentic samples of D-mannose, D-galactose and D-xylose in solvent (B). The fractions 1-12 containing same sugar were combined together and concentrated to give D-mannose. It was recrystallised from aqueous methanol, $[\alpha]_D^{25}$ + 12.9° (in water, C, 1.7 of par 100 ml of solution). The following two derivatives were prepared:

(i) D-Manrose Phenyl hydrazone

Found	Given[76] (Lit.).
m.p. 196-198°	199-200°

(ii) D-Mannose p.n.glycosylamino benzoic acid

The derivative was prepared according to the recent method of Ellis[77].

Found	Given (Lit.)[77]
m.p. 179-181°	182°

The fraction 14-25 were mixed and concentrated to give D-galactose. It was recrystallised from aqueous methanol, $[\alpha]_D^{25}$ + 77° (in water, C, 0.5g per 100 ml of solution). The following derivatives were prepared :

(i) D-Galactose Phenyl Hydrazine

Found	Given (Lit.)[78]
m.p. 154°-155°	154°-155°

(ii) N-p-nitrophenyl-D-Galactosylamine

In a micro test-tube, galactose (45 mg), p-nitroaniline (45 mg), one drop of glacial acetic acid and four drops

of methanol-water(8:1 v/v) were taken. The mixture was boiled for 8 minutes and kept over-night in a refrigerator. The crystalline product was filtered, washed with cold ethanol, ether and dried in vacuum. It melted at 217-18° after recrystallisation from methanol. Lit[79] m.p. 219°.

The fraction 30-38 containing same sugar were combined together and concentrated to give D-xylose. It was recrystallised from aqueous methanol, $[\alpha]_D^{30}$ + 18.5° in water, C, 1.15 %), m.p. 144-45°. The following derivative was prepared.

(i) D-xylose phenyl osazone derivative

The osazone of the sugar was prepared as given on page 43 m.p. 160-161° resembling to an authentic sample.

III.18 (c) Thin-layer chromatography

The plates were prepared from slurry of silica Gel G in 0.1N solution of boric acid and the spots of hydrolysate alongwith benzene:acetic acid : methanol (1:1:3)[80] and air dried. These plates were sprayed with aniline hydrogen phthalate reagent on heating them at 120° in an oven three spots corresponding to D-galactose, D-xylose and D-mannose were observed.

III.19 QUANTITATIVE ESTIMATION OF MONOSACCHARIDES

The polysaccharide (200 mg) was hydrolysed with 2N sulphuric acid (35 ml) for 24 hours on a boiling water-bath and neutralised with barium carbonate. Ribose (20 mg) was added to it. The hydrolysate was applied on Whatman No.1 filter paper alongwith the guide strip. After developing in solvent (C), the strips corresponding to the sugars were cut

with the help of guide spots and eluated. The eluate was oxidised with periodate and the quantity of the monosaccharide estimated as described on page .

TABLE - 3

Sugar	Volume of alkali* used in (ml)			Corresponding amount of sugar (in mg)		
	A	B	C	A	B	C
D-Galactose	3.36	4.22	2.76	0.97	1.21	0.80
D-Mannose	13.60	16.98	11116	3.92	4.89	3.21
D-Xylose	3.18	4.02	0.62	0.98	1.20	0.79
D-Ribose	1.96	2.42	1.60	0.59	0.72	0.48

* Strength of sodium hydroxide = N/124.8.

Assuming complete recovery of D-Ribose the above results indicate that in the polysaccharide, D-mannose, D-galactose and D-xylose in the molar ratio of 4:1:1.

XII.20 GRADED HYDROLYSIS OF THE POLYSACCHARIDE

The polysaccharide (150 mg) was dissolved in 0.05-N-sulphuric acid (30 ml). The hydrolysis carried out over a boiling water-bath. The hydrolysate, taken out at various intervals, were examined chromatographically, without removal of sulphuric acid using solvent (B) for the purpose of irrigation of the paper. Results are given in the table -4.

TABLE - 4

Time (in minutes)	Sugars identified	No.of other spots
10	Galactose (Faint)	-
15	Galactose+xylose(Faint)	-
30	Galactose + Xylose	-
45	Galactose + Xylose	Two spots
60	Galactose+Xylose + Mannose(Very faint)	Three spots
120	Galactose + Xylose + Mannose (Faint)	Three spots

Time (in minutes)	Sugar identified	No.of other spots
180	Galactose + Xylose + Mannose	Three spots
240	Galactose + Xylose + Mannose	Three spots

D-galactose was found to liberate first followed by the liberation of D-Xylose and D-mannose. The easy release of D-galactose leads to the conclusion that galactose is present as terminal group,and not in the main chain of the polysaccharide. The release of D-xylose before the release of D-mannose shows that most of xylose units are present as terminal group and mannose units are present in the main chain and formed the backbone of the polysaccharide.

III.21 METHYLATION OF THE POLYSACCHARIDE

The polysaccharide (10g) was dissolved in minimum quantity of water and was methylated first by the method due to Parikh, Ingle and Bhide[55] followed by Purdie's method[56] as usual described on page 47.

The partly methylated product was brownish mass(7.8g). $-OCH_3$,36.4%, $[\alpha]_D^{25}$+55.6° (in chloroform,C,1.5 per 100 ml of solution). The partly methylated polysaccharide was further methylated by Purdie's method[56] as given on page 48 . The fully methylated polysaccharide was obtained as a deep brownish coloured product (6.8 g) found $-OCH_3$, 44.06 $[\alpha]_D^{25}$+40° (in chloroform,C,1.5 g per 100 ml of solution).

III. 22 HYDROLYSIS OF THE METHYLATED POLYSACCHARIDE AND IDENTIFICATION OF METHYLATED SUGARS

The hydrolysis of methylated polysaccharide was

carried by slight modification of method due to Bouveng et al[82]. The methylated polysaccharide (100 mg) was dissolved in 85% formic acid (20 ml) and rest of the process was carried out as described on page 49 .

After separation on Whatman No.1 filter paper in solvent (A), the chloroform chromatogram of syrup showed five spots after spraying with aniline hydrogen phthalate and drying at 120°. The R_{TMG} value of each methylated sugar was calculated in solvent (A) and was compared with that, given in literature as shown in the following table -5.

TABLE - 5.

Methylated sugars identified	Solvent (A)	
	R_{TMG} found	R_{TMG} 58, 83 given
2,3,4,6-Tetra-O-methyl-D-galactose	0.90	0.88
2,3,4,tri-O-methyl-D-xylose	0.92	0.94
2-O-methyl-D-xylose	0.39	0.38
2,3 -di-O-methyl-D-mannose	0.45	0.44
2,3,6-tri-O-methyl-D-mannose	0.82	0.81

III.23 QUANTITATIVE ESTIMATION OF METHYLATED SUGARS

III.23.1 The methylated polysaccharide (300 mg) was hydrolysed as given above. To the hydrolysate glucose (60 mg) was added and then neutralised with barium carbonate.

The chromatogram was developed by descending method using solvent (D) as described on page 94.

The sugars were estimated by alkaline hypoiodite method as given on page 50 .The results obtained are given in the table-6.

TABLE - 6

Fraction & its sugars	Volume of 0.1N hypo used (in ml)			Corresponding amount of sugars (in mg)		
	A	B	C	A	B	C
A. 2,3,4,6-tetra-O-methyl-D-galactose	0.80	1.28	0.94	0.872	1.395	1.024
B. 2,3,4,tri-O-methyl-D-xylose	0.50	0.80	0.60	0.435	0.696	0.522
C. 2-O-methyl-D-xylose	0.60	0.96	0.70	0.438	0.700	0.511
D. 2,3,di-O-methyl-D-mannose	0.92	1.46	1.10	0.874	1.387	1.045
E. 2,3,6-tri-O-methyl-D-mannose	2.56	4.12	3.00	2.611	4.202	3.006
F. Glucose	0.58	0.94	0.70	0.522	0.846	0.630

The above results corresponded to an average molar ratio between A, B, C, D and E as 2:1:1:2:6. The methylated sugars were calculated as the methyl ethers of anhydrohexose and pentose units, i.e. $C_6H_{12}O_5$ and $C_8H_{16}O_5$ for mono- and tri-O-methyl-D-xylose and $C_{10}H_{20}O_6$, $C_9H_{18}O_6$, and $C_8H_{16}O_6$ for tetra-, tri- and di-O-methyl sugars respectively. An average recovery of the methylated polysaccharide was found to be 98.88% assuming 100% recovery of glucose.

III.23.2 CHARACTERISATION OF METHYLATED SUGARS

The methylated polysaccharide was hydrolysed according to the method of Garegg and Lindberg[84] as described on page 52. The mixture of different methylated sugars was resolved into five fractions on Whatman No.3 filter paper using solvent(D) Strips containing different individual methylated sugars were eluted with water. The eluates were concentrated separately under reduced pressure and marked as fractions I.

II, III, IV and V.

III.23.3 Fraction I

Solid, R_{TMG} in solvent (A), 0.89 , found OMe- 51.3% calculated for tetramethyl hexose, OCH_3, 52.54 %, $[\alpha]_D^{25}$ + 123° (in water,C,0.5%), Lit.[85] for 2,3,4,6-tetra-O-methyl-D-galactose $[\alpha]_D^{16}$ + 142° → +117° (equil.) (in water,C,1.1%), m.p. 70-72°. It gave red colour with aniline hydrogen phthalate. Its treatment with alcohlic aniline gave 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine, m.p. 188-89°.

III.23.4 Fraction II

Syrup, it could not be recrystallised. The R_{TMG} in solvent (A) 0.95, optical rotation of sugar was found to be $[\alpha]_D^{18}$ + 19.2 (in water, C, 0.38 %), Lit[86] is $[\alpha]_D^{15}$ + 203°, OMe found 54.98 %, calculated for $C_8H_{16}O_5$ is 55.35%)

The anilide of the sugar was prepared as given on page 53 The melting point of the anilide was found to be 95-96°, $[\alpha]_D^{22}$- 80° (in ethanol,C,1.8%) Lit.[86], m.p. 120°,$[\alpha]_D$-84→+ 47° (in ethanol) and Lit.[87] m.p. 91°. The methoxyl value of the derived anilide was found to be 33.6% ($C_{14}H_{21}O_4N$ requires, OMe 34.3%).

The sugar in this fraction was thus identified as 2,3,4-tri-O-methyl-D-xylose.

III.23.5 Fraction III

Solid, R_{TMG} in solvent (A), 0.39, OMe, 18.92% calculated for mono methyl pentose, $C_6H_{12}O_5$, OMe, 18.90 %, m.p. 132-133°, $[\alpha]_D^{25}$ -25° (in water,C, 2.3 %) Lit.[88] m.p. 135-37°.

$[\alpha]_D$ - 23→+ 35° (in water) Lit.[89] m.p. 132-33°; $[\alpha]_D$ -24→ +36° (in water). It formed 2-O-methyl-D-xylose anilide on treatment with ethanolic aniline, m.p. 122-24°, $[\alpha]_D^{25}$- 10° (in ethyl acetate;C,0.9 %).

On acetylation as usual method it formed a crystalline compound 2-O-methyl-D-xylose; 3,4-di-acelate, m.p. 76-77°, $[\alpha]_D^{25}$- 39° (in chloroform,C,3.0%) Lit.[89] m.p. 78-79° $[\alpha]_D$- 38° (in chloroform). Thus the above confirmed that the methylated sugar III, is 2-O-methyl-D-xylose.

III.23.6 Fraction IV

Syrup, R_{TMG} in solvent (A), 0.54, $[\alpha]_D^{26}$ -16.6° (in water, C, 1.8%), found OMe, 29.37% calculated for dimethyl; OMe, 29.8%, $[\alpha]_D$- 16.0° (water) in Lit.[90].

The sugar (100 mg) was dissolved in pyridine. It was finally washed with water and dissolved in chloroform. The insoluble portion was filtered out and the solvent from the filtrate was evaporated in a vacuum desicator. The crude product was recrystallised from ether, m.p. 190-192°, $[\alpha]_D^{26}$ + 63° (in chloroform, C, 1.5%). Lit[81], for 1,4,6-p-nitro-benzoate of 2,3-di-O-methyl-D-mannose, m.p. 194° and $[\alpha]_D$ +65° (in chloroform).

III. 23.7 Fraction V

Syrup, R_{TMG} in solvent (A), 0.82, found OMe, 41.3% calculated for tri-methyl hexose; OMe 41.9% $[\alpha]_D^{25}$- 12.5° (in water, C, 1.6 g per 100 ml of solution). Lit[92] for 2,3,6-tri-O-methyl-D-mannose, $[\alpha]_D^{25}$- 10° in water.

The syrup (100 mg) was dissolved in dry pridine (6 ml)

and treated with p-nitrobenzoyl chloride (35.0 mg) for 45 minutes at 60-70° and left over-night at room temperature. A saturated solution of sodium bicarbonate was added dropwise until no effervescene occured. After adding water (15 ml), the product was extracted with chloroform. The extract was dried over sodium sulphate, excess of solvent was taken off in vacuum and crystallised from petroleum ether, m.p. 186-87°, $[\alpha]_D^{26}$ + 32° (in chloroform,C,0.5%). Lit[93,94] for 1,4-bis-p-nitrobenzoate of 2,3,6-tri-O-methyl-D-mannose, m.p. 187-88° and $[\alpha]_D$+ 33.0°. The syrup (100 mg) was oxidised with bromine water and the product crystallised from aceton-petroleum ether, m.p. 80-81° Lit[61] for 2,3,6-tri-O-methyl-γ- (+) mannolactone, m.p. 82-83°. The lactone (75 mg) was boiled under reflux in alcohol with phenyl hydrazine (45 mg). It was then refluxed with little amount of animal charcoal in ethanol and filtered. On cooling, a crystalline product was obtained which was recrystallised from ethanol, m.p. 128-30° $[\alpha]_D^{25}$ - 20.5 (in water, C, 0.8%). Lit[95], for 2,3,6-tri-O-methyl-D-mannonic acid phenyl hydrazide, m.p. 131°,$[\alpha]_D$ - 20° (in water).

III.24 PERIODATE OXIDATION OF THE POLYSACCHARIDE

III.24(a) Liberation of Formic Acid[96] & Estimation of end groups

The polysaccharide (300 mg) was dissolved in water (50 ml) and in the solution, potassium chloride (0.5 g) and 0.25M sodium metaperiodate (60 ml) were added. The volume was made upto 140 ml with water. In a blank experiment potassium chloride (0.5 g) and 0.25M sodium metaperiodate (60 ml) were

diluted to 140 ml with water. The oxidation was carried out in a dark at room temperature as described on page 55 . The aliquots of 5 ml were taken and were titrated for liberated formic acid against N/124.8 sodium hydroxide solution using methylated as indicator. Results are given in table - 7.

TABLE - 7

Time (in hours)	Volume of alkali used in (ml.)	Corresponding amount of formic acid (in mg)	Total formic acid (in mg)
8	1.32	0.506	14.168
16	1.40	0.536	15.02
24	1.54	0.590	16.52
36	1.72	0.659	18.46
48	1.88	0.720	20.178
60	2.00	0.766	21.46
72	2.04	0.782	21.896
84	2.04	0.782	21.896

The data shows that 0.782 mole of formic acid was liberated (72 hours) per 100 g of the polysaccharide. The amount of formic acid liberated (72 hours) corresponds to 24.9% of anhydrohexose and pentose units present as end groups. The titre value of alkali at 48, 60 hours indicated that one mole of formic acid was liberated per 683.9% and 643.05 g of the polysaccharide respectively.

III.24 (b) Consumption of Sodium Metaperiodate[97]

The polysaccharide (300 mg) was dissolved in water (70 ml) to which 0.25M) sodium metaperiodate (40 ml) was added and the total volume was made upto 120 ml with water. A blank was also prepared with 0.25M sodium metaperiodate

(40 ml) diluted to 120 ml with water. The periodate oxidation was carried out at room temperature as described on page 57 . The liberated iodine from 2 ml aliquots of mixture and blank were titrated against 0.0404N sodium thiosulphate solution at various intervals using starch as indicator. The reading with the polysaccharide were substracted from the corresponding reading of controlled experiment to get the titre values. The results are given in table - 8.

TABLE - 8

Time (in hours	Volume of Hypo used (in ml)	Periodate consumed (in mg)	Total periodate consumption(in mg)
8	1.00	4.322	259.33
16	1.12	4.841	290.45
24	1.32	5.705	342.32
36	1.42	6.137	368.25
48	1.60	6.915	414.94
60	1.72	7.434	446.06
72	1.84	7.953	447.18
84	1.86	8.039	482.36
96	1.86	8.039	482.36

The amount of periodate consumed (84 hours) corresponds to the consumption of 0.7513 moles of periodate per 100 g of the polysaccharide. After 84 hours periodate oxidised solution (10 ml) was hydrolysed with 2N sulphuric acid (Page 58). The hydrolysate examined by paper chromatographically for the presence of D-galactose, D-xylose and D-mannose but the chromatogram did not indicate the presence of any of the three sugars.

III.25 PARTIAL ACID HYDROLYSIS OF POLYSACCHARIDE

The polysaccharide (2 g) was suspended in water

(500 ml) in a three necked flask and stirred mechanically and the same procedure was adopted as described on page 58.

III.25.1 Examination of the Precipitate

The precipitate was hydrolysed and identified similarly as described on page 59. The chromatograms, showed three spots corresponding to R_g value of D-galactose, D-xylose and D-mannose which were confirmed by co-chromatography with their authentic samples.

III.25.2 Examination of the Hydrolysate

Paper chromatographic analysis of the hydrolysate over Whatman No.1 filter paper using solvents (A) and (B) and aniline hydrogen phthalate as a spraying reagent produced seven spots thereby indicating the presence of seven sugars.

III.25.3 Separation of Oligosaccharide

The syrup was dissolved in minimum quantity of water. It was separated by paper chromatography as described on page 59. The sugars were crystallised from ethanol and five fractions of oligosaccharide and two fractions of monosaccharides were obtained.

III. 25.4 Examination of Fraction I and Identification of Mannotriose

$R_{Man\ 1}$ 0.07 and R_{Gal_2} 0.37 in solvent (C) and (G) respectively, R_{Gal_2} 0.21 in solvent (F). The sugar was crystallised from ethanol, m.p. 168-69° $[\alpha]_D^{30}$ - 18.8 (in water, C,1.5%). It reduced Fehling's solution and Tollen's reagent.

The complete acid hydrolysis with 2N sulphuric acid,

subsequent neutralisation with barium carbonate and examination by paper chromatography with an authentic sample only one monosaccharide, D-mannose was obtained. The equivalent weight of the sugar was found to be 264.8 by hypoiodite method[57]. Partial acid hydrolysis of sugar with 0.5N hydrochloric acid at 100° for 10 minutes resulted in formation of mannose and mannobiose which were identified by co-chromatography with their authentic samples.

Periodate oxidation of the sugar revealed that 2.10 moles of formic acid were liberated and 5.3 moles of periodate were consumed per mole of sugar. The sugar was completely hydrolysed with emulsin suggesting that mannose units were linked through β-glycosidic linkages.

On the basis of above results the sugar was identified to be mannotriose i.e. β-mannose pyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-mannopyranose, which was further confirmed by its physical constants of it as shown in table-9.

TABLE - 9

Constants	Found	Reported	References
m.p.	168-69°	133-139° and 214-15°(anhydrous)	(83, 98, 99)
Optical rotation	$[\alpha]_D^{30}$ -18.8	$[\alpha]_D$ -15°→- 26°	(100, 101)
R_{Glu} in solvent (G)	0.37	0.33	(102)
and Solvent(F)	0.21	0.22	(63, 64)

III.25.3 Examination of Fraction II and Identification of Epimelibiose

R_{Man} 0.15, 0.24, 0.35 in solvent (A), (B) and (C) respectively. The sugar was recrystallised from ethanol, m.p. 200-02°, $[\alpha]_D^{32}$ + 120.5° (in water, C, 0.49 per 100 ml of solution).

Acid hydrolysis of the sugar with 2N sulphuric acid and neutralisation of the hydrolysate with barium carbonate followed by paper chromatography with solvent (C), revealed the presence of galactose and mannose in the oligosaccharide which was further confirmed by co-chromatography with an authentic sample.

The quantitative estimation by the method of Hirst and Jones[54] showed the molar ratio to be 1 : 1 between the two sugars in the oligosaccharide. The equivalent weight as determined by hypoiodite method[57] was found to be 174.2.

The periodate oxidation studies corresponded to the consumption of 5.24 moles of metaperiodate and liberation of 3.2 moles of formic acid per mole of the oligosaccharide. Thus there is 1→6 linkage between galactose and mannose units. As the oligosaccharide could not be hydrolysed with emulsin it shows that galactose and mannose have α-linkage between them.

On the basis of above observations the sugar was identified as epimelibiose, 6-O-α-D-galactopyranosyl-D-mannopyranose. Its identity was further confirmed by preparing its osazone, m.p. 172° and co-chromatography with an authentic sample. The observed constants of the sugar were compared with those reported in literature as shown in the table - 10.

TABLE - 10

Sugar or derivative	Constant	Found	Reported	References
Epimelibiose	m.p.	200-02°	201 - 02° & 202 - 03°	(103,104)
Epimelibiose	Optical rotation	$[\alpha]_D^{32}$ +120.5° (in water)	$[\alpha]_D$ +120.9° $[\alpha]_D$ +120.9→ →124.6° (in water)	(104,105)
Epimelibiose	R_{Glu} in Solvent(G)	0.60	0.59	(64)
Osazone	m.p.	172°	175 - 76°	(105)

III.25.6 Examination of Fraction III and Identification of 6^2-α-Galactosyl mannobiose

R_{Man} 0.07 and 0.16 in solvent (C) and (B) respectively. The sugar was recrystallised from 90% ethanol. The paper chromatography revealed only one spot. R_{Glu} in solvent (G) 0.33., m.p. 226-27° and $[\alpha]_D^{32}$ + 98.5° (in water, c.0.5g per 100 ml of solution). It reduced Fehling's solution and Tollen's reagent.

The complete acid hydrolysis with 2N sulphuric acid, neutralised with barium carbonate and chromatographic examination showed the presence of galactose and mannose. The quantitative estimation by the method of Hirst and Jones[54] showed that galactose and mannose constituents the oligosaccharide in the molar ratio of 1:2. The equivalent weight was found to be 262.8 by hypoiodite method[57].

The periodate oxidation studies showed that one mole

of the oligosaccharide consumed 6.30 moles of metaperiodate and liberated 3.18 moles of formic acid. Partial acid hydrolysis revealed the presence of manncbiose, epimaltbiose besides galactose and mannose.

From the above observations, the sugar was identified to be α-Galactopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-D-mannopyranose. The observed datas were found to be close agreement with the reported values in literature as shown in table - 11.

TABLE - 11

Constants	Found	Reported	References
m.p.	226 - 27°	228 -29°	(91, 103)
Optical rotation	$[\alpha]_D^{32}$ + 98.5°	$[\alpha]_D^{25}$ + 93.3° → +98.8	(91, 103)
R_{Glu} in solvent(C)	0.33	0.32	(64)

III.25.7 Examination of Fraction IV and Identification of Manncbiose

R_{Man} in solvents(A) and (B) and (C) were found to be 0.28, 0.47 and 0.33 respectively. The sugar was recrystallised from methanol, m.p. 203°, $[\alpha]_D^{30}$ - 11.6° (in water, C, 1.3 g per 100 ml of solution).

Acid hydrolysis with 2N sulphuric acid, followed by neutralisation with barium carbonate and subsequent examination by paper chromatography showed the presence of mannose units only. The equivalent weight was determined by hypoiodite

method[57] and was found to be 174.8.

The periodate oxidation studies showed the consumption of 4.22 moles of periodate with the liberation of 2.14 moles of formic acid per mole of the sugar. The sugar was completely hydrolysed with emulsin showing the presence of β-linkage between the mannose units which was also confirmed by the negative optical rotation of the sugar.

Thus the oligosaccharide is a disaccharide compose of D-mannose units linked through β-glycosidic linkage. The sugar was identified to be manncbiose i.e. 4-O-β-D-mannopyranosyl-D-mannopyranose which was also confirmed by preparing osazone derivative, m.p. 205° and co-chromatography with an authentic sample. The constants of sugar are given in table - 12.

TABLE - 12

Sugar or Derivative	Constants	Found	Reported	References
Manncbiose	m.p.	203°	202-204°	(63,65. 83,103)
Manncbiose	Optical rotation	$[\alpha]_D^{30}$ -11.4°	$[\alpha]_D$ - 7° → - 9°	(63, 83, 101, 103)
Manncbiose	R_{Glu} in solvent (F) and (G)	0.52 0.66	0.52 0.65	(63, 64)
Manncbiosazone	m.p.	205°	203-06°	(63)

III.25.8 Examination of Fraction V and Identification of Rhodymenabiose

$R_{Xylobiose}$ values were 1.98 and 1.04 in solvent (E) and (F) respectively. Recrystallised from methanol, m.p.

191°, $[\alpha]_D^{22}$ - 21° (in water,C, 2.92 g per 100 ml of solution).

Acid hydrolysis of the sugar V with 2N sulphuric acid and neutralisation with barium carbonate, followed by paper chromatographic analysis in solvent (C), reveals the presence of xylose only. The molecular weight was determined by hypoiodite method[57], 296. molecular weight calculated for xylobiose, $C_{10}H_{18}O_9$, 282.

The periodate oxidation studies showed the consumption of 3.24 moles of metaperiodate with the liberation of 1.18 moles of formic acid. The sugar was completely hydrolysed with emulsin showing the presence of β-linkage. Its identity was further confirmed by preparing its phenyl-osazone derivative, m.p. 197 - 98°, $[\alpha]_D^{22}$ + 48° (in pyridine, C, 2.0%, calculated for $C_{22}H_{28}O_7N_4$, N 12.18, found 12.30%.

Constants of sugar were compared with those reported in literature as shown in table - 13.

TABLE - 13

Sugar or derivative	contants	Found	Reported	References
Rhodymenabiose	m.p.	191°	192 - 93°	(53)
Rhodymenabiose	$R_{xylobiose}$ in solvent(B)	1.98	1.97	(52)
-do-	Optical rotation	$[\alpha]_D^{22}$ -20.4	$[\alpha]_D^{22}$ -18.4± 0.6°	(52)
3-O- -D-xylo-pyranosyl-D-xylose-phenyl osazone	m.p.	197-98°	194 - 96°	(53)
-do-	Optical rotation	$[\alpha]_D^{22}$ +48°	$[\alpha]_D$ + 47°	(53)

III.25.9 Examination of Fraction VI and identification of D-galactose.

R_G . 0.81 in solvent (C), R_{Man}. 0.62 and 0.80 in solvent (A) and (B) respectively. The sugar was crystallised from aqueous methanol, $[\alpha]_D^{32}$ + 80.7° (in water, C, 1.0%). It was identify to be D-galactose by co-chromatography with an authentic sample.

III.25.10 Examination of Fraction VII and identification of D-mannose.

R_F . 0.12 in solvent (A) and R_G . 1.09 in solvent (C), $[\alpha]_D^{30}$ + 12.9° (in water, C, 2.1%). The sugar was identify to be D-mannose by co-chromatography with an authentic sample.

REFERENCES

1. (a) Chopra, R.N.; Chopra, I.C.; and Nayer, S.L., 'Glossary of Indian Medicinal plants'; 172(1956).

   (b) Chopra, I.C., Chopra, R.N, and Varma, S.S.; 'Supplimant to Glossary of Indian Medicinal Plants'; Page No. 72, (1969).

2. Howes, F.N., 'A Dictionary of useful and everyday plant and their common names', Page No. 18, First published (1974)

3. Fernandes, O., and Alfageme, C. Rev. Sanid. Hig. Pub. 11, 525-35 (1936).

4. Caroline Sherman Lanford, Beatrice, Frinkelstein, and Sherman, H.C.; J Nutrition 21, 172-7 (1941).

5. De Moura, F.A.,Compose (Univ. Sao: Paulo, Brasil). Brasil med. 61, No 20/21/22. 197-99 (1946).

6. Sadana, J.C., and Bashir Ahmad, J. Sci.,and Research (India) 8B, No.2. 35-9 (1949) CA.43, 2708 h

7. Hazel E. Munsell, Louise, Williams, Louise P. guild, Lucille, T. Kelley and Robert S Harris.; Food Research 15, 421-38 (1950) CE, CA. 45,3523 h.

8. Chung-Ching Su, Tanching. Ma, and Yam-Hui Lin (Natl. Taiwan Univ., Taipei). CHung Hso Nung Yeh Hsa Hsueh Hui Chih (1-2) 33-4. (1963).

9. Chung-Ching Su, and Ta-Hsiu Liao (Natl. Taiwan Univt. Taipei) Chung Hso Nung Yeh Hsa Hsueh Hui Chih (1-2) 42-3. of Preceding Abstr. (1964).

10. Agot, A. (Sta. Hosh. Auxiolas, Canst. C.M.R.S Jouey-en-Josas, Fr.). Bull. Soc., Sci., Hyg. Aliment (1968)

56 (1-3), 27-41 (Fr.)

11. Knapp, Furn F., Nicholas, Harold. Phytochemistry, 8 (10), 2091-3 (1969) (Eng.)

12. Mai, G.A., Lih, S.S. Chung Kuo Nung Yeh Hua Hsueh Hui Chih (Special Issue), 1-17 (Eng.) (1970).

13. Shantha, N.S.; Siddappa, G.S. J. Food Sci.,(1970), 35 (1) 72-4 (Eng.)

14. Maki, Mitsuaki., Sato, Yukio., Nosaigaku Zasshi (1969) 28 (6), 406-10 (Japan).

15. Simmonds,N.W., Nature 173, 402-3 (1954).

16. Andrews R.S., and Pridham, J.B, Phytochemistry,6 (1), 13-18 (1967) (Eng.).

17. Bottier. R.A, Fr. I, 169, 727, Jan.5, 1959.

18. Buzarova,V.I., Sb. Tr.Leningv. Inst. Sov. Torgovli. No.23, 71-80 (1964) (Russ).

19. Buckley, S.H, (United fruit Co Norwood, Mars).. Phenolics Norm.Dis. Fruits Veg. Proc. Symp 4th Norwood, Mars(1964) (1-6), discussion 16-18 (Pub.1965) (Eng.).

20. James F. Eheart and Branche S. Mason, J.Amer.Diet. Ass. 50 (2), 130-2. (1967) (Eng.)

21. James F.G. Hawkins, E.G. Jones, J.K.N. and Young G.T.; J.Chem.Soc. 390-4(1940) (Eng.).

22. Barrios, M.L. Gonzale, M.A. J. Ag.Univ. P.R.(1971), 55(2), 263-4 (1971) (Eng.)

23. Sanyal, A.K., Gupta, K.K., and Choudhary, N.K., Arch. Intern. Pharmacody. 182,(3-4) 393-400 (1964)(Eng.)

24. Bolivarde Mora, Clara.; Rosas M., Ana M., Technologia. 12 (64), 42-8 (1970 (Span).

25. Ferrell, William J.; Drouillard, Mark., Physiol.Chem.Phys. (1970),2 (2), 168-70(Eng.)

26. Lalla, B.S., and Johar, D.S., Current Sci., (India), 24, 92-3. (1955).

27. Lallas B.S; and Johar, D.S., Current Sci.,(India).

28. Kri Korian, A.D. (State Univ. of New York, Stony Brook N.Y) Econ. Bot.,22 (4) 385-9 (Eng.) (1968).

29. Hady Lopez Soryan, Salutridady asistencia social(Havana), 46, 140-83 (1943).

30. Philip, L. Harris and Gev. Poland. Food Research, 2, 311-19 (1937).

31. Crois bois, Micklale, Mazliah P., Food Sci. Technol.Proc. Inst. Congr. Int (1962) (Pub. 1969), 285-91 (Fr.).

32. Crosbois, Michale, and Mazliak, P. (Sta. Froid, Ballevene), Paris) Fruits Paris 19 (2) 55-9(1964).

33. Pereira, J.R., Bastos, R.S. and Zyngier, Z. Arch. Intern. Pharmacology 144(1/2) 144-50 (1963) (Eng.)

34. Sanyal, A.K., Banerjee, C.R. and Das, P.K., Arch. Intern. Pharmacology, 155(1),244-48 (1965)(Eng.)

35. William E. Scott, Hazel. N. Mckay, F.S; Schaffer and Thomas D. Fontaine. J. Clin. Invest. 28, 899-902(1949).

36(i) Vincenzo. Carelli, Paolo Marchini, and Aldo Tucci.; Ann. Chein (Rome) 45, 1126-32 (1955).

(ii) Vincenzo, Carelli, and Paolo Marchini, Ibid, 1133-45(1955).

37. Bartholomew Nagy, Vincent Modzeleski and sister Marxy, Murphy, T.J. Phytochem., 4 (6), 945-50 (1966) (Eng.).

38. Jain, Sneh.R . Planta Med. 17(1) 98 (1966) (Eng.).

39. Knapp Furn F. Nickolas, Harold J. Steroids(1970), 16(3), 329-5 (Eng.)

40. Knapp, Furn F., Nicholas, Harold J. Mol.Cryst.Liquid Cryst. 6(3-4), 399-28(1970) (Eng.)

59. Mikes, O.; 'Laboratory Hand-Book of Chromatographic Method's, Ist Ed. Van Nostrand.P. 71 (1966).

60. Rizvi, S.A.I.; D. Phil. Thesis, University of Allahabad, (India) (1968.).

61. Andrews, P., Hough, L. and Jones, J.K.N.; J.Am. Chem. Soc.; 74 , 4029 (1952).

62. Hamilton, J.K., Partlow, E.V. and Thompson, N.S.; J. Chem. Soc., 215 (1958).

63. Aspinall, G.O., Rashbrook, R.B. and Kessler, G.; J.Chem. Soc., 215 (1958).

64. Meier, H.; Acta Chem. Scand.; 14 749 (1960).

65. Courtois, J.E.; Petek, F. and Kade. T.; Bull. Soc. chem. Bio., 40 , 2031 (1958).

66. Morgan, K. and O. Neil, A.N.; Cand. J. Chem. 37 ,1201,(1959).

67.(a) Tewai, S.N.; J. Anal. Chem. 176 604 (1960).

(b) Wilson, C.M., Anal. Chem. 31, 119 (1959).

68.(a) Marier, J.R. Boulet. MC; J. Dairy Sci.,42, 1390(1959).

(b) Dubois, M., Gilles, K.A., Hamilton, J.K. Rebers, P.A., Smith, F., Anal. Chem., 28 , 350 (1956).

69. Carego, A.B., J. Org. C hem. 30 , 924 (1965).

70.(a) Lederer, E. and Lederer, M.; 'Chromatographic Method's, Ist Ed. P. 88 (1966).

(b) Mikes, O.; 'Laboratory Hand-book of Chromatography', Elsvier's P. 166 (1955).

71. Trvelyan, W.E., Proctor, D.P. and Harrison, J.S.,Nature 166 , 444 (1950).

72. Ellis, G.P.; Chem. Ind., 902 (1966).

73. Belcher, R. and Godbert, A.L., 'Semi-micro-quantitative Organic analysis', 2nd Ed., P. 164 (1954).

74. Barker, S.A., Foster, A.B., Siddiqui, I.R. and Stacey, M., Talanta, 1 , 216 (1958).

75. Partridge, S.M.; Biochem. J. 42 238 (1948).

76. Isbell, H.S., and Frush, H.L.; 'Methods in Carbohydrate Chemistry' (Ed. Whistler, R.L) Academic Press, Inc. Vol. II, P. 117(1363).

77. = 72.

78. Mesler, L., 'Methods in Carbohydrate Chemistry', (Ed. Whistler R.L.), Academic Press Ic.Vol.II,P.117,(1963).

79. Misaki, A. and Smith, F., Agr. Food. Chem. 10, 104 (1962).

80. Pastuska, G.P J. Anol. Chem. 179, 427 (1961).

81. Smith, F. and Montegomery, R., 'The Chemistry of Plant Gums and Mucilage', Am. Chem.Soc., Monograph series, Reinhold Publishing Corporation, New York, P. 134 (1959).

82. Smuveng, H.O., Kissling, H., Lindberg, Band MC-Kay, JE., Acta Chem. scand., 16 615 (1962).

83. Tyminski, A. and Timell, T.E.; J. Am Chem. Soc.,82,2823(1960).

84. Garegg. P.J. and Lindberg. B.; Acta Chem.Scand.,14, 871 (1960).

85. (a) Melchor, R. Fildes, J.E. and Hutten, A.J. Analyt. chem. Acta, 13 , 16 (1955).

(b) Rafique, M.C. and Smith, F., J. Am. Chem.Soc., 76,2221(1954).

(c) White, E.V. and Rao, P.S., J. Am. Chem. Soc. 75,2617(1953).

86. Chanda, S.K.; Hirst, E.L., Jones, J.K.N., Percival, E.G.V., 1289 (1950).

87. Cifonelli, J.A. and Smith, F., Anal Chem.26, 1132 (1954); Ibid., 77 , 1984 (1935).

88. Percival, E.G.V, and Willox, I.C., J.Chem.Soc.,1608 (1949).

89. Robertson, G.J, Speedie, T.H, j. Chem. Soc.,824 (1934).

90. Robinson, G.L.; J. Chem. Soc., 330 (1934).

91. Whistler, R.L. and Durso, D.F.; J. Am Chem. Soc., 74, 5140 (1952).

92. Haworth, W.N., Hirst, E.L. and Plant, M.M.T.; J.chem. Soc., 1354 (1931).

93. Hirst, E.L. and Jones, J.K.N., J.Chem.Soc., 1278 (1948).

94. Whistler, R.L., 'Methods in Carbohydrate chemistry', Academic Press, Vol.V, P. 332 (1965).

95. Andrews, P., Hough, L. and Jones, J.K.N., J.Chem.Soc, 2744(1952).

96. Brown, P., Malmall, T.G., Hirst, E.L., and Jones, J.K.N.; J.Chem. Soc 28 (1948).

97. Hough, L. and Powell, D.B., J. Chem.Soc, 16 (1960).

98. Aspinall, G.O., Rashbrook, R.B. and Kessler, G.; J.Chem. Soc., 215 (1962).

99. Goldstein, I.J. and Whelan, W.I.; J. Chem. Soc., 170(1962).

100. Jones, J.K.N., and Painter, T.J.; J. Chem. Soc., 669, (1957).

101. Oyaw, M.O. and Tisall, T.S., Canad.J. Chem., 38, 1957(1960).

102. Perila, O. and Bishop, C.T.; Canad. J.Chem.39 ,815 (1961).

103. Mallary, R.A.; "Oligosaccharides", International Series of Monographs on pure and Applied Biology, Biochemistry Division, Vol. 4, Pergamon Pres, New York.P, 51(1965).

104. Anderson, M.E., Hough, L. and Painter, T.J.; J.Chem. Soc., 2519 (1958).

105. Whistler, R.L. and Durso, D.F. J. Am. Chem.Soc.,73.4189(1958).

106. Boisnave,Francis,R.C.,Streichan berger,Gilles, F.R. Lachat, Paul R.M., Ger. Fr. Appl. (1968).

CHAPTER - IV

CHEMICAL EXAMINATION OF TWO FLAVONOIDS AND AN ANTHOCYANIN FROM THE FRUITS OF GARCINIA GUMMIFERA LINN.

IV.1 In the present chapter chemical examination of two flavonoids and an anthocyanin from the fruits of Gardenia gummifera Linn., has been described.

Gardenia gummifera Linn., commonly known as 'Dika mali', belongs to the family Rubiaceae[1], is a small unarmed nearly glabrous shrub with resinous buds. Leaves, sessile or subsessile, 1½ - 2½ inches in long, obovate, acute or obtuse, shining, base obture, acute or cordate sometimes puberulous beleath. Stipules, connate, truncate or mucronate. Flowers, 1 - 3 together, subsessile, calyx, pubesent, lobes short, ovate, acute. Corolla, white turning to yellow, its tube 1-2 inches long, glabrous or pubesent; limb 1-3 inches, across; lobes 5 obleng, abtuse. Fruit 1 - 1½ inches in long, ellipsed or obleng and smooth pericarp thin, placenta, 4 - 5. Flowers during March and April.

The plant is found in Bandelkhand region and its distribution in Southwards from chota Nagpur and Bombay. It is tropical and subtropical shrub, cultivated, Ornamentally.

The fruit is eaten. The gum is collected and sold together with a similar substance yielded by G.lucida. Gum of G. gummifera[2] is antispasmodic, Carminotive, antiseptic, stimulant, anthelmintic. It is also used in veterinary medicine to keep off flies from sores. Its ornamentally flowers are often perfumed. Some species used in local dyeing[3]. Gardenia seeds are useful in enhance healing of soft tissue[4].

IV.2 The details of research work reported in the literature on this plant is given in tabular form on the next page.

Genus	Species	Constituents	Parts	References
1. Gardenia	-	Stable yellow food colouring agent	-	(1979)[5]
2. Gardenia	-	Colour of natureal dye (Capa Jasmine dye)	Fruits	(1976)[6]
3. Gardenia	-	Food colouring agent (Yellow, green & blue pigments)	Seeds	(1978)[7]
4. Gardenia	-	Extraction of Orange-yellow pigment from defatted gardenia	-	(1975)[8]
5. Gardenia	Florida	Mannitol	Foliage leaves & fruits	(1919)[9]
6. Gardenia	Florida (grandiflora)	Crocin & crocetin (colouring matter of saffran group)	Fruits	(1922)[10]
7. Gardenia	Grandiflova (Japanese wongshy)	Crocin, mannitol & a plant hormone	Seeds	(1944)[11]
8. Gardenia	Jasminoides and grandiflora	10-acetyl geniposide & picrocinic acid	Fruits	(1976)[12]
9. Gardenia	Jasminoides & grandiflora	Three new glucosides Gardenoside, shanzhiside-Me deacetyl asperulosidate, geniposide, genipengentiobioside	Fruits	(1974)[13]
10. Gardenia	Jasminoides & grandiflora	Tarchnoside, tarenna gracilipes, gardenoside, geniposide, & geniposidic acid	Cultured cells tissue	(1981)[14]
11. Gardenia	Jasminoides	Oleophilic natural dye (lipophilic dye for foods cosmetics)	-	(1979)[15]
12. Gardenia	Jasminoides	Crocin glucoside	-	(1950)[16]
13. Gardenia	Jasminoides	Gardenoside(8,10-dihydrogeloganic acid) & scandoside-Me ester.	Fruits	(1974)[17]

(Continued)

Genus	Species	Constituents	Parts	References
14. Gardenia	Jasminoides	Genipin & genipin 1-β-D-gentiobioside (2-new iridoid glucoside)	Fruits	(1973)[18]
15. Gardenia	Jasminoides	Gardenoside, genipaside (Two new iridoid glucoside)	Fruits	(1969)[19]
16. Gardenia	Jasminoides	Crocin extraction	Fruits	(1974)[20]
17. Gardenia	Jasminoides	Shanzhiside, (a new iridoid glucoside)	Fruits	(1970)[21]
18. Gardenia	Jasminoides	Genapin-1-β-gentiobioside (New iridoid glycoside)	Fruits	(1970)[22]
19. Gardenia	Jasminoides	Nonacosane, β-sitosterol, D-Mannitol.	Fruits	(1964)[23]
20. Gardenia	Lucida (Dikamali gum)	Hexacosyl p-coumarate (a new phenolic ester)	Leaf bud exudate	(1980)[24]
21. Gardenia gum	Dikamali gum	5,7,3',4',-tetrahydroxy 6,8-dimethoxy flavone	Gums	(1977)[25]
22. Gardenia gum (Dikamali gum)	-	Gardenin, Demethyl tangeretin, nevadensin 5,7,dihydroxy- 6,3,4,5 tetra methoxy flavone	-	(1971)[26]
23. Gardenia gum (Dikamali gum)	Lucida	Gardenin A, B, C, D & E	Resinous exudate	(1970)[27]
24. Gardenia gum	-	A mix. of p-coumaric esters of higher alcohals (C_{22}- C_{26})	-	(1979)[28]
25. Gardenia	Lucida	D-Mannitol	Root bark	(1966)[29]
26. Gardenia	Gummifera	Gleenonic aldehyde, erythrodiol, 19-α-hydroxy erythrodiol-sitosterol, D-mannitol.	Stem bark	(1977)[30]

(Continued)

Genus	Species	Constituents	Parts	References
27. Gardenia	Turgida	D-Mannitol, hexaacetyl-D-mannitol, D-mannitol-hexabenzoate, gardenin A, B & E, oleanotic acid, α-Amyrin and β-sitosterol.	Roots	(1979)[31]
28. Gardenia	Turgida	D-Mannitol.	Exudation	(1925)[32]
29. Gardenia	Turgida	β-sitosterol, D-mannitol, oleanolic acid methyl ester, gypsogenic acid methyl ester, & hederagenic methyl ester	Wood & bark	(1973)[33]
30. Gardenia	Posbergii	Three new flavones	Rad studate	(1979)[34]
31. Gardenia (African varieties)	Pomodora & vogelii	D-mannitol	-	(1975)[35]
32. Gardenia	Latifolia	Mannitol & sitosterol.	-	(1969)[36]
33. Gardenia	Latifolia	D-mannitol, sitosterol oleanolic, siaresinolic, spinosic acids and hederagenin	Stem b-ark	(1975)[37]
34. Gardenia	Latifolia	3-episia resinolic acid (anew triterpene acid)	Bark	(1975)[38]
35. Gardenia (African varieties)	Jovis, tomantia	D- Mannitol	Root-bark	(1974)[39]
36. Gardenia	Fructus	Crocin, Crocetin	-	(1954)[40]
37. Gardenia	Fructus	Drugs	-	(1976)[41]
38. Gardenia	Angusta	Pharmacology	Roots & leaves	(1936)[42]

From the survey of literature it seems that the root, leaves, stem, bud and fruits, gum of genus have been extensively examined for various plant products. Since no study on anthocyanin and flavonoids compounds from the fruits of Gardenia gummifera, therefore, it is worthwhile to investigate thoroughly the plant fruits of G. Gummifera for their chemical constituents.

IV.1 EXTRACTION AND ISOLATION OF FLAVONOIDS AND ANTHOCYANIN FROM THE FRUITS OF GARDENIA GUMIFERA

The fruits of Gardenia gummifera were purchased locally and identified for their authenticity in the Botany department of D.V. Postgraduate College, ORAI,(Bundelkhand university).

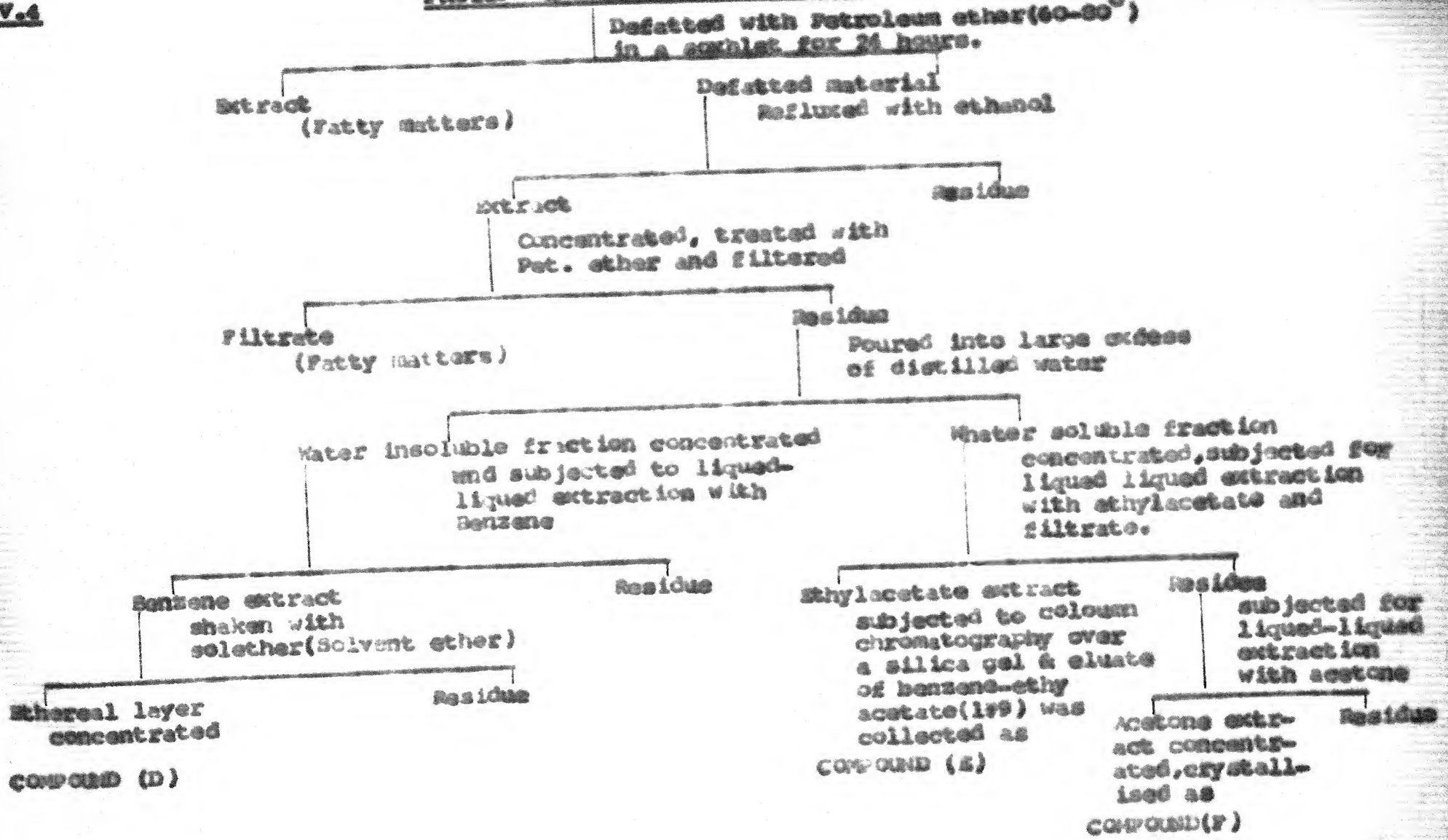
Dried and crushed seeds (2 kg) were defatted with Petrolium ether (60-80^{0}) in a soxhlet extractor. The defatted material was extracted with ethanol (95%) on a steam-bath in several lots. The total extract was concentrated at reduced pressure to a reddish brown viscous mass. It was refluxed with petroleum ether (60-80^{0}) to remove the fatty material and resulting residue, still viscous mass, was poured into 1 litre of distilled water with vigorous stirring. The water soluble and insoluble fractions were separated by separating funnel and successively subjected to liquad-liquad extraction, using petroleum ether, benzene, ethyl acetate and acetone respectively.

The benzene fraction of water insoluble part was

shaken with solvent ether in a separating funnel, several times. The etherial layer was separated and the solvent was evaporated whereupon a light-yellow substance was obtained. The purity of the substance was tested on TLC which give a single spot. This pal yellow mass on crystallisation from acetone methanol (1:1), gave an yellow compound, D, m.p.349°. The ethyl acetate extract of water soluble part was subjected to column chromatography over a silica gel G. The benzene-ethyl acetate (1:9) eluate of the colum yielded a dark yellow coloured compound (E), having m.p. 150°.

The alcohlic extract of water soluble fraction was refluxed with acetone (B.D.H.) in different lots. The whole extract was reduced to very small (20 ml) volume whereupon a solid mass (Orange-red) was obtained. The acetone acetate part gave a single spot on TLC using ethyl acetate-acetone (9:1) and B...1 (4:1:5 v/v) systems, showed a single compound in the extract. The compound (F) was crystallised from methanol having m.p. 300°

IV.4


FRUITS OF GARDENIA GUMMIFERA
Defatted with Petroleum ether(60-80°) in a soxhlet for 24 hours.
Extract (Fatty matters)
Defatted material Refluxed with ethanol
Extract
Residue
Concentrated, treated with Pet. ether and filtered
Filtrate (Fatty matters)
Residue
Poured into large excess of distilled water
Water insoluble fraction concentrated and subjected to liquid-liquid extraction with Benzene
Water soluble fraction concentrated, subjected for liquid liquid extraction with ethylacetate and filtrate.
Residue
Benzene extract shaken with solether(Solvent ether)
Residue
Ethereal layer concentrated
COMPOUND (D)
Ethylacetate extract subjected to coloumn chromatography over a silica gel & eluate of benzene-ethy acetate(1:9) was collected as
COMPOUND (E)
Residue subjected for liquid-liquid extraction with acetone
Acetone extract concentrated, crystallised as
COMPOUND(F)
Residue

SECTION - A

IV.5 CHEMICAL STUDY OF COMPOUND (D)

Benzene extract of water insoluble fraction of ethanolic extract of fruits of Gardenia gummifera afforded a compound (D), m.p. 349° and molecular formula, $C_{15}H_{10}O_5$. It was isolated from the fruits of Gardenia gummifera as described on page 131 and was shown to be single entity by paper chromatography and responded to the following colour reactions :

(i) It gave pink colour in Shinoda reduction[43], but did not give pink colour with hydrochloric acid alone.

(ii) It gave intense yellow colour with characteristic fluorescene[44,45] by conc. sulphuric acid.

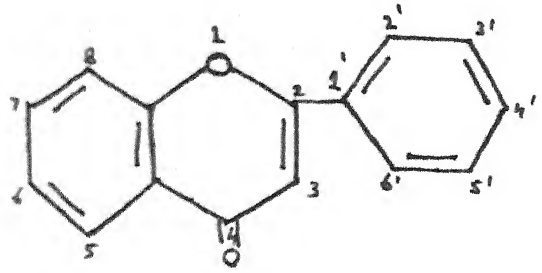
(iii) It gave an yellow orange colour with ethanolic ferric chloride[46].

(iv) It produced yellow colour with liquad ammonia which showed yellow fluorescene in UV light[47].

(v) A yellowish colour was obtained on treatment with sodium hydroxide solution, which was stable on heating[48].

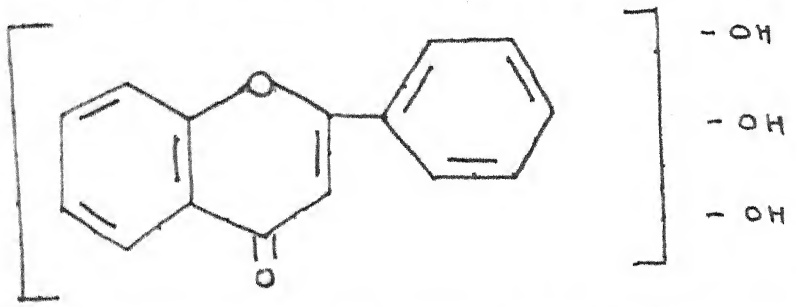
(vi) No change in colour was observed on addition of vanillin hydrochloric acid reagent to the compound (D).

The above reactions suggest that the compound (D) is a flavone derivative possessing following skeleton.



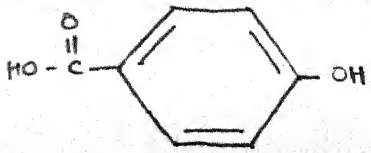
It gave negative Molisch's test indicating thereby the aglycone nature of the compound.

The presence of this skeleton of pale yellow coloured compound (D) also supported by the absorption maxima of the compound at 269 nm and 336 nm. The skeleton accounts only for $C_{15}H_{10}O_2$, which suggests that the remaining three oxygen items may be present as three hydroxyl groups in the aglycone. The compound formed a trimethyl ether and triacetate on methylation and acetylation respectively, confirming the presence of three hydroxyl groups in it. Thus the compound may be represented as below :



The relative position of these three hydroxy groups have been assigned on the basis of various colour reactions, degradation and spectral studies of the compound.

The compound on oxidation with neutral potassium permagganate gave a compound identified as p-hydroxybenzoic acid. This reaction shows that one hydroxyl group compound (D) ⟶

Potassium permanganate
Oxidation ⟶ 

p-hydroxy benzoic acid

is present at position -4' of B ring of the compound. This was further confirmed by the following facts :

(i) When an excess of sodium bicarbonate was added to the solution (Shinoda reduction) of the compound a blue colour[49,50], was obtained, showing the presence of free hydroxyl group at position -4.

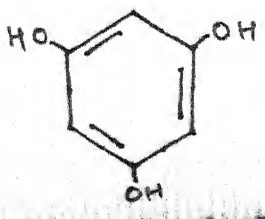
(ii) An ethanolic compound showed a bathochromic shift of 40 nm of Band 1 (from 336 to 376 nm) by the addition of fused sodium acetate, indicating the presence of hydroxy group at position 4' or 3[51]. The possibility of hydroxyl group at position -3 was eliminated by the fact that the yellow colour given by the compound with aqueous sodium hydroxide was stable on heating[48].

(iii) A bathochromic shift of 56 nm of band 1 (from 336 nm to 392 nm) without a decrease in relative intensity was observed by the addition of sodium ethoxide to the ethanolic solution of the compound. This shift is diagnostic[53,54] for the presence of free hydroxyl group at position -4.

(iv) A single well defined peak (266) of band II of the compound in ethanol also confirmed the presence of 4 - substituent in the B-ring[52].

The compound (D) on fusion with potassium hydroxide gave[55] a compound identified to be phloroglucinol. This degradation showed the presence of free hydroxyl group at positions -5 and 7.

Compound (D) $\xrightarrow[\text{Potassium hydroxide}]{\text{5\% Aqueous}}$



Phloroglucinol.

The presence of free hydroxyl group at position -5 was further confirmed by the following facts :

(i) The compound (D) gave an orange-red colour with Dimroth's reagent (acetyl pyroborate)[56].

(ii) The compound gave bright yellow colour with methanolic zirconium oxychloride showing the presence of free hydroxyl group at position -5[57]. The colour did not change on addition of citric acid showing the absence of hydroxyl group at position -3 in the molecule[57].

(iii) When the compound (I) in acetone was treated with a solution of boric acid and citric acid in acetone, it give a yellow colour with yellowish green fluorescence. This shows the presence of methoxyl or hydroxyl group at position -5[58].

(iv) An ethanolic solution of the compound gave green colour with the ethanolic Ferric chloride[46].

(v) Bathochromic shifts of 46 nm in Band I (from 336 nm to 382 nm) and of 9 nm in Band II (from 269 nm to 278 nm) were observed by the addition of a few drops of ethanolic aluminium chloride to the ethanolic solution of the compound. This showed a free hydroxyl group at position -5 of the aglycone[59,60].

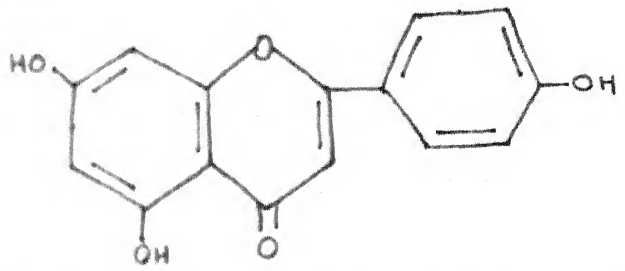
The presence of free hydroxyl group at position -7 of the compound (I) was supported by the flollowing facts :

(i) Pink colour was given by the aglycone with vanillin hydrochloride reagent, indicating the presence of free 5,7, -di-hydroxy grouping[61] in the molecule.

(ii) A bathochromic shift of 9 nm of Band II (from 269 nm to 278 nm) was observed on addition of a little fused sodium acetate to the ethanolic solution of the compound, confirming the

presence of free hydroxyl group at position -7[51]. The compound also did not give any precipitate with neutral lead and acetate[62] showing the absence of ortho-dihydroxy grouping.

Hence, on the basis of observations, the compound (D) has been assigned the following structure 4', 5, 7 -trihydroxy flavone (Apigenin).



IV.6 EXPERIMENTAL:

It was soluble in ethanol, methanol, acetone, pyridine and insoluble in petroleum ether, benzene and water. It gave all positive tests, characteristics of flavonoids, as described on page 133.

IV.7 CHROMATOGRAPHY OF THE COMPOUND

The purity of the compound was checked on Whatman No.1 filter paper when a single spot was observed in each case using following solvent system :

	R_f
(i) n-Butanol-acetic acid-water (4:1:3 v/v)	0.97
(ii) Ethanol saturated with water	0.96
(iii) m-cresol-acetic acid-water(50:2:48 v/v)	0.88

IV.8 ELEMENTAL ANALYSIS OF THE COMPOUND

Found	Calculated for $C_{15}H_{10}O_5$
C = 66.62 %	66.60%
H = 4.0%	3.70%

IV.9 ACETYLATION OF THE COMPOUND

The compound (50 mg) was acetylated with acetic anhydride (50 ml) and pyridine (3.0 ml). The reaction mixture was left overnight and poured in ice-cold water with constant stirring. It was filtered, washed wll with water, dried and recrystallised from methanol to yield acetyl derivative, m.p. 187 - 88°.

IV.10 DETERMINATION OF ACETYL PERCENTAGE

The acetyl percentage in the acetylated derivative was determined by the method of Wisenberger[63] as described by Godbert and Belcher[64].

Found	Calculated for $C_{15}H_7O_5(COCH_3)_3$
Acetyl group = 31.95%	= 32.57 %

IV.11 METHYLATION OF THE COMPOUND

The compound (40 mg) was taken in dry acetone (20 ml) and was methylated with dimethyl sulphate (5 ml) and anhydrous potassium carbonate (1.0g) by refluxing it on a water-bath for 24 hours. The reaction mixture was cooled, filtered and poured over crushed ice, whereupon a yellow mass was settled down. It was filtered, washed and recrystallised from ethanol, m.p. 158°.

IV.12 DETERMINATION OF METHOXYL GROUP PERCENTAGE

The methoxyl percentage in the methylated derivative of compound was determined by the method of Belcher, Wilcox and Nutten[65].

Found	Calculated for $C_{15}H_7O_2(OCH_3)_3$
Methoxyl group = 28.68%	= 29.80%

IV.13 POTASSIUM PERMANGANATE OXIDATION OF THE METHYL ETHER

The methylated compound (20 mg) was oxidised with neutral potassium permanganate solution under reflux for 6 hours. The reaction mixuture was cooled and the excess of manganese dioxide was destroyed by adding sodium bisulphate to it. The resulting solution was acidified with dilute hydrochloric acid, whereupon a white compound was separated out. It was filtered and crystallised from ethanol, m.p. 178°. It was identified to be anidic acid by its mixed melting point and co-chromatography with an authentic sample.(R_g 0.37 in n-butanol saturated with ammonia; spray bromophenol blue solution).

IV.14 UV AND VISIBLE SPECTRA OF THE COMPOUND

UV and visible spectra were recorded on Beckman Model DV spectrophotometer.

Solution and reagent	max.(nm)	Shift
(i) Compound+Ethanol	269,336	- -
(ii) Compound+Ethanol+NaOAc	278,386	9, 40
(iii) Compound+Ethanol+$AlCl_3$	278,382	9, 46
(iv) Compound+Ethanol+NaOEt	277,392	8, 56

IR SPECTRUM OF COMPOUND (D)

Following prominent peaks (cm^{-1}) were observed in the IR spectrum of the compound :

3442, 3289, 1660, 1625, 1590, 1580, 1355, 1205 842, and 710.

SECTION - B

IV.15 CHEMICAL STUDY OF THE COMPOUND (E)

An yellowish brown compound (E), had m.p. 190°, isolated from the ethanolic extract of Gardenia gummifera Linn as described on page 131 . The molecular formula was found to be $C_{24}H_{26}O_{11}$. It had R_f value 0.41 in n-Butanol: Acetic acid: water (4:1:5 v/v) system. On refluxing with 7% ethanolic sulphuric acid it gave a water insoluble aglycone, having molecular formula $C_{18}H_{16}O_7$. The compound responded to a positive Molish test showing that the presence of a glycoside. The extract nature of the glycoside was confirmed by the identification of and chracterisation of the aglycone and sugar moiety obtained on acid hydrolysis of the compound.

IV.16 STUDY OF THE AGLYCONE

An yellow aglycone, $C_{18}H_{16}O_7$ had m.p. 204°. Ethanolic solution of the aglycone gave the following colour reactions and chemical tests.

(i) Various shades of red colour were obtained when it was treated with.

(a) Mg + Hcl[43]

(b) Zinc + Hcl[66]

(c) Na/Hg + Hcl[45]

(ii) It produced a dull green colour on treatment with ethanolic ferric chloride.

These colour reactions suggest that the compound may be a derivative of flavone or flavanone.

(iii) When it exposed to vapours of ammonia liqued on a

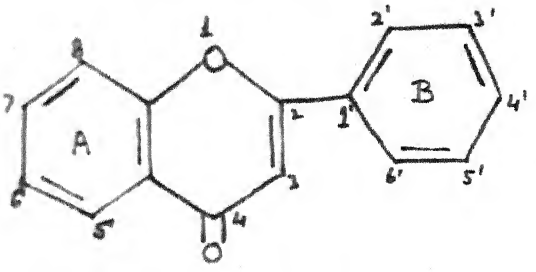
filter paper, the compound turned to darkyellow colour and showed fluorescence under UV[47] light.

(iv) A dark yellow brown colour was obtained on treatment with aqueous sodium hydroxide.

(v) It did not respond to a positive reaction with 2,4- Dinitro Phenyl hydrazine reagent[67].

(vi) It could not be reduced with Sodium Borohydride showing the absence of flavanone skeleton[68].

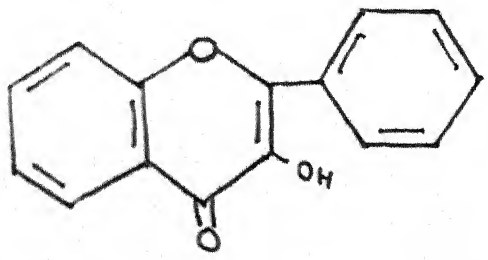
From the above reaction it is obvious that this compound is a flavone derivative and should have the following skeleton.



(vii) It showed a positive reaction with ethanolic Boric acid and sodium acetate[69].

(viii) It responded to a positive reaction with Zirconium oxychloride in presence of citric acid[57].

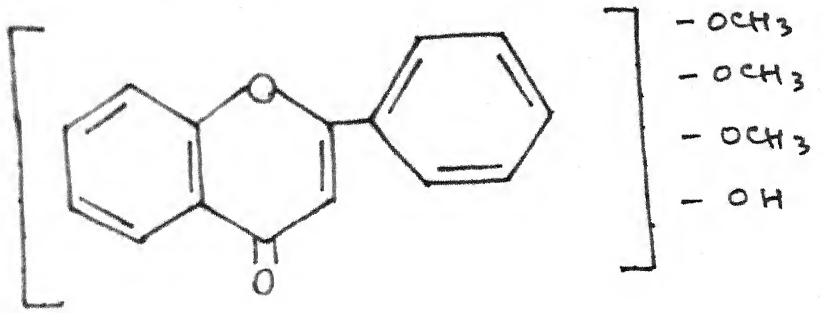
These reactions indicates that there is a hydroxy group at position -3. Thus the skeleton of the compound is designed as 3-hydroxy flavone or flavanol having the following skeleton.



This skeleton of the compound is further confirmed by

the absorption maxima at 255 nm and 369 nm[47,52]. Thus three oxygen items, two in the benzopyrone nucleous and one as -OH at positive -3 have been accounted for the compound(I). The compound (I) was found to contain three methoxyl groups by Zeisel's method, IR peaks at 2850[70] cm^{-1} and 1185 cm^{-1} [71,72] and formed a diacetate showing the presence of two free hydroxyl groups in its structure.

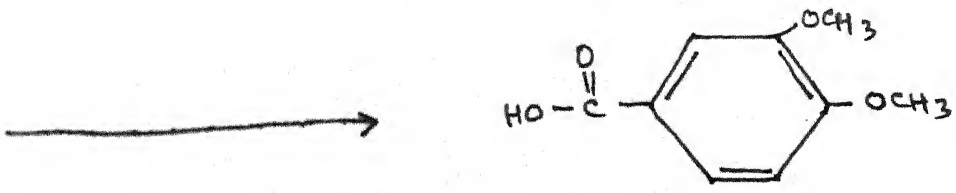
Thus the aglycone can be represented as, and this accounts for all the seven oxygen atoms in it.



The relative position of methoxyl groups and of the hydroxyl group have been determined by the degradation studies of the compound and its colour reactions.

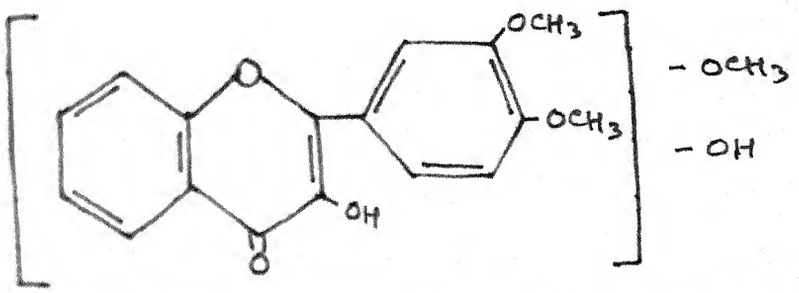
When compound was oxidised with neutral potassium permanganate, veratric acid was one of the products isolated from the oxidation mixture.

Aglycone of the compound —Potassium permanganate oxidation→

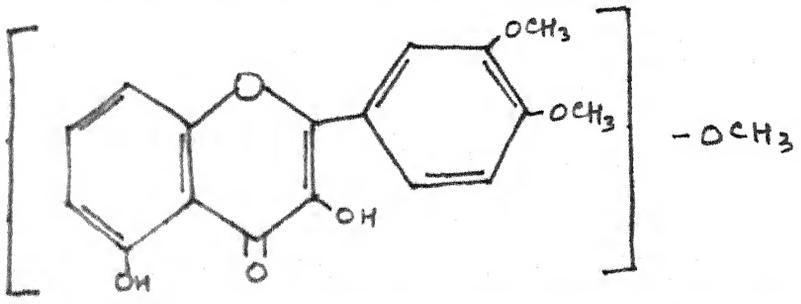


Veratric acid

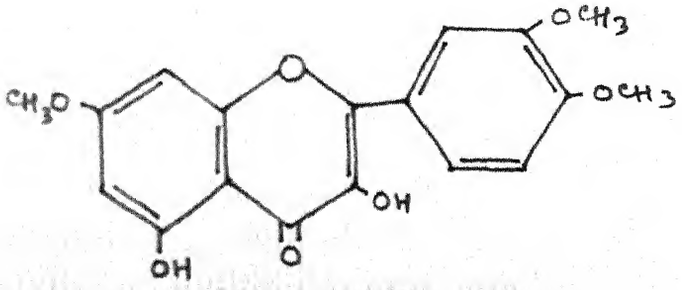
Presence of this compound clearly indicates the position of two methoxy group at -3' and - 4' positions. Thus the structure may be represented.



The aglycone of compound (X) gave a green colour with ethanolic ferric chloride[46] and responded to a positive test with ethanolic boric acid in presence of citric acid[47,49], showing the presence of a free hydroxy group at position -5. Thus the structure may be written as follows :-

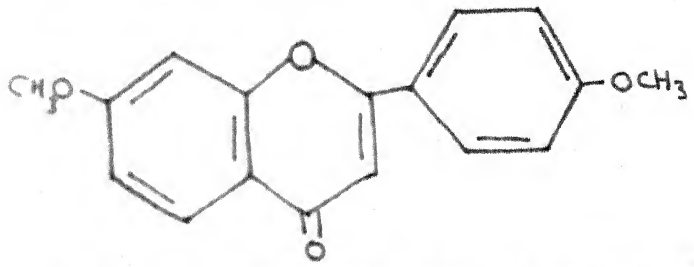


The possible position for the remaining methoxyl group appears to be - 7 position in the ring A, which is indicated by the absence of a positive colour reaction with vanillin hydrochloric acid[51] reagent. Thus the structure of the compound as follows :



This structure of the aglycone is supported by the following colour reactions and spectral properties of the compound.

(i) The presence of -7, 4' - dimethoxy flavanol skeleton is shown by peak at 1604 cm^{-1} in the IR spectrum of the compound[73, 74].



(ii) The absence of the hydroxyl group at position -7 is shown by the fact that the compound did not shown any bathochromic shift (max. 255 nm) in the UV region on addition of fused sodium acetate to it ethanolic solution[75].

(iii) The presence of free hydroxyl group in position -5 has been shown by the following facts :

(a) When aglycone of the compound was treated with ethanolic $AlCl_3$ on a filter paper, it produced fluorescence under U.V. light[77].

(iv) The presence of free hydroxyl group at position -3 has been shown by the fact that a bathochromic shift of 46 nm (max. changed from 360 nm to 415 nm) was observed on addition of 1% ethanolic $AlCl_3$ to the ethanolic solution of the compound[76].

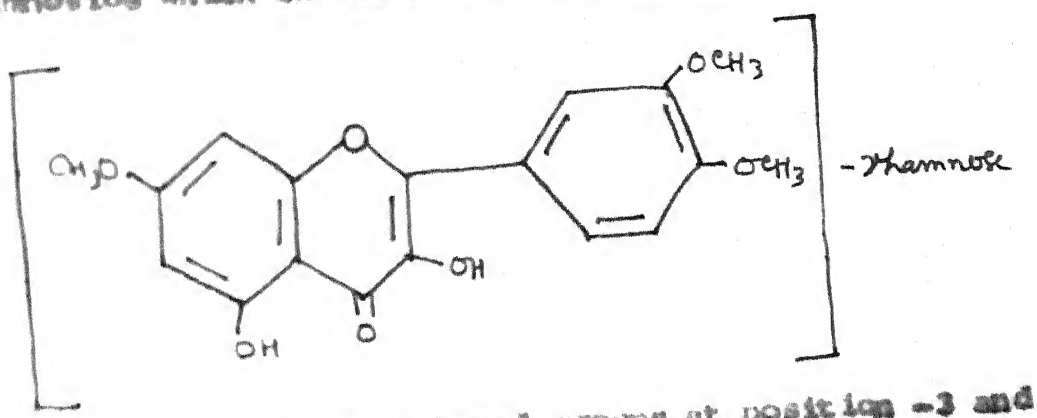
(v) The absence of free hydroxyl group at -3' and -4' position is confirmed by the fact that the compound did not show any bathochromic shift (max. 369 nm) in visible reagion on addition of boric acid and sodium acetate[78] to its ethanolic solution.

IV.17 IDENTIFICATION OF THE SUGAR

The solution so obtained from the hydrolysis of the compound (E), was found to respond to positive Molish's test and reduced Fehling solution. It gave a spot with R_g value 0.36 in n-Butanol:acetic acid: Water(4:1:5 v/v) system and formed an osazone, having m.p. 190 with Phenyl hydrazine reagent, suggesting the sugar to be rhamnose. This was confirmed by its co-chromatography with an authentic sample.

IV.18 STUDY OF ORIGINAL COMPOUND (E) AND POSITION OF GLYCOSIDIC LINKAGE

From the above discussion it is obvious that compound(E) is a rhamnoside which can be represented as below :

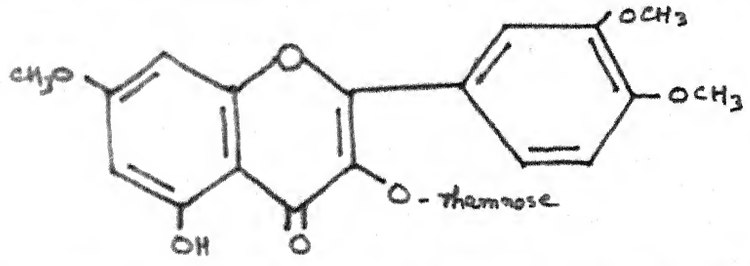


Since there are free hydroxyl groups at position -3 and -5 hence it is clear that sugar residue could be linked in either of these two positions. (-3 or -5 positions) A clue to the position of the sugar linkage has been obtained by comparing the properties of the glycoside with that of the aglycone.

It has been mentained earlier that the aglycone responded to a positive reaction with sodium borate[69] and also zirconium oxy-chloride in presence of citric acid[57] showing the presence of free hydroxyl group at position -3. However under similar

condition. Glycoside did not respond to these tests from which it appears that sugar is linked at the position -3 of the aglycone residue.

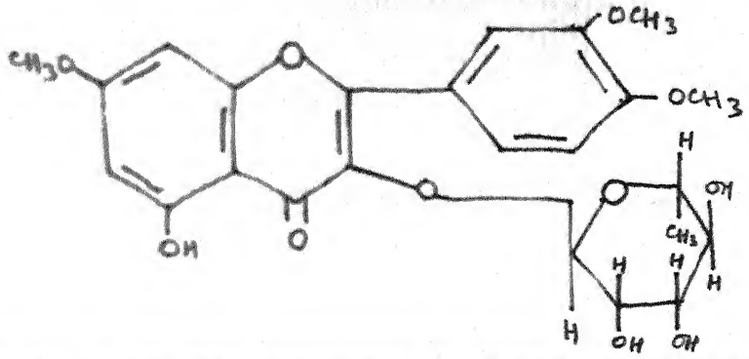
On methylation of the compound (E) with diazomethane and subsequent hydrolysis of the methylated derivative, a compound was obtained which was identified to be quercetin 7,5, 3', 4' -tetra methyl ether, m.p. 192° [80] and the spectral properties (max. at 253 nm and 362 nm)[75,79]. This confirm the attachment of the sugar at position-3 of the aglycone.



The glycoside did not reduce Fehling's solution nor gave a positive test with aniline hydrogen phthalate, reagent, suggesting that aldehyde group of the sugar is not free[79] and is involved in glycosidic linkage.

The glycoside on periodate oxidation consumed 2:1 moles of periodate and produced 1:2 moles of formic acid per mole of the compound, showing that the sugar was present in the pyranose form. The glycoside got hydrolysed with emulsin showing that the sugar was linked with the aglycone through the β -linkage[86].

The glycoside (E) thus can be represented as follows :



This structure of the compound(E) clearly explains the following spectral correlations :

(1) The compound was found to be stable in 0.002M sodium ethylate showing the bathochromic shift of 12 nm (max. changed from 357 nm to 369 nm) in the visible region of spectrum, showing the absence of free hydroxyl group at positions -3 and -4'[81].

(2) The presence of peak between 835 cm^{-1} to 810 cm^{-1} in the I.R. spectrum of the compound is in confirmation with the pyranose structure of the sugar[81,82,83].

EXPERIMENTAL

IV.19 ISOLATION AND PURIFICATION

An yellowish brown coloured compound (E), was isolated and purified as described on page 131 . It was recrystallised from chloroform-methanol(9:1) mixture, m.p. 190°.

IV.19.i HOMOGENEITY OF COMPOUND (E)

The homogeneity of the compound was checked by paper chromatography on Whatman No.1 filter paper using following solvent systems :

(i) n-Butanol-acetic acid-water (4:1:5 v/v)
(ii) Acetic acid-conc HCl-water (30:3:10 v/v)

Spot was developed by exposing to the vapours of ammonia. A yellow single spot was observed which showed fluorescence under U.V.light.

ELEMENTAL ANALYSIS

Found	Calculated for $C_{24}H_{26}O_{11}$
C = 57.8%	C = 58.8%
H = 4.8 %	H = 5.3 %

IV.20 HYDROLYSIS OF THE COMPOUND

The compound (300 mg) were dissolved in minimum quantity of ethanol and in 100 ml round bottom flask. (50ml) of the 7% ethanolic sulphuric acid were added and the reaction mixture was refluxed on a water bath for 10 hours.

The hydrolysed was cooled, solvent distilled off, diluted with water and filtered. The precipitate was dried in vacuum crystallised from acetone: chloroform (5:5 v/v) and finally recrystallised from methanol to yield a yellow coloured

compound (aglycone) m.p. 205°. The filtrate obtained after removal of the aglycone was neutralised with barium carbonate, filtered and concentrated under reduced pressure to a syrupy mass.

IV.21 IDENTIFICATION OF SUGAR

The syrup obtained after the hydrolysis of the glycoside was examined paper chromatographically using n-Butanol-acetic acid-water (4:1:5 v/v) solvent system. The developed chromatogram was airdried, sprayed with aniline hydrogen phthate and on heating at 120° for 10 minutes, one spot, R_g value 0.35 in n-Butanol - acetic acid-water(4:1:5 v/v) system was observed which corresponded to rhamnose. This was further confirmed by co-chromatography with an authentic sample.

IV.22 EXAMINATION OF THE AGLYCONE

M.P. 205°, soluble in ether, acetone, ethanol and methanol, sparingly soluble in chloroform but insoluble in petroleum ether, benzene and carbon tetrachloride.

HOMOGENEITY OF THE AGLYCONE

The purity of the aglycone was checked by paper chromatography on Whatman No.1 filter paper using n-Butanol-acetic acid-water (4:1:5 v/v) and phenol saturated with water in each case a single spot was observed.

ELEMENTAL ANALYSIS

	Found	Calculated
C =	62.4%	62.8%
H =	4.5 %	4.65%

IV.23 ACETYLATION OF THE AGLYCONE

The aglycone was acetylated using acetic anhydride and pyridine by the usual method of acetylation. The acetyl derivative was crystallised from acetone. M.p. 180°.

IV.24 DETERMINATION OF ACETYL PERCENTAGE

The percentage of the acetyl groups in the acetylated product was determined by the method of Wiesenberger[63] as described by Melcher and Godbert[64].

Found

Acetyl percentage = 20.1%

Calculated for $C_{18}H_{14}O_7(COCH_3)_2$

= 20.6%

IV.25 METHYLATION OF AGLYCONE

The aglycone (4 0 mg) was taken in dry acetone (20 ml) and was methylated with dimethyl sulphate (5 ml) and anhydrous potassium carbonate (1.0g) as usual method as described on page 138.

DETERMINATION OF METHOXYL GROUP PERCENTAGE

The determination of methoxyl group in the aglycone was done by Zeisel's method as described by Melcher Fiede and Nutten.

Found

methoxy groups

= 26.3%

Calculated for $C_{15}H_4O_4(OCH_3)_2$

methoxyl groups

= 27.03%

IV.26 POTASSIUM PERMANGANATE OXIDATION OF THE AGLYCONE

The aglycone (20 mg) were treated with aqueous potassium permanganate under reflux for four hours. The reaction mixture was cooled and excess of manganese dioxide destroyed by adding sodium bisulphate to it. On addition of hydrochloric acid a

a white precipitate was separated out which was crystallised from acetone, m.p. 120 -21°. It was identified to be veratric acid, Lit.[84] m.p. 120 -22°.

IV.27 METHYLATION OF COMPOUND (E)

The Glycoside (25 mg) was methylated by using (20 ml) ether solvent of diazomethane by the usual method. The methylated derivative was crystallised from chloroform having m.p.168-70°.

IV.28 PERIODATE OXIDATION OF THE COMPOUND (E)

The glycoside (20mg) dissolved in 25 ml ethanol and 25 ml distilled water were treated with 25 ml of .1M sodium metaperiodate solution. A blank was also prepared similarly.

The periodate oxidation was estimated by titrimetric method of Jones etal[85] 15 ml. aliquots of the solution was taken out from the reaction mixture.

Molecular weight of the compound	= 2190
For 15 ml aliquots of the solution 0.1N sodium hydroxide consumed	= 0.35 ml
0.1N Hypo consumed	= 0.9 ml
For each mole of the glycoside moles of periodate consumed	= 2.1
moles of formic acid liberated	= 1.2

IV.29 SPECTRAL STUDIES

All the spectral measurements were taken using Beckman model Dus spectrophotometer and absolute ethanol as the solvent system.

Solvent and Reagent		max n m	Shift n m	
(A) Glycoside (E)				
(i)	Ethanol	268, 357	-	-
(ii)	Ethanol/$AlCl_3$	380	-	23
(iii)	Ethanol/NaOEt	369	-	12
(iv)	Ethanol/$NaOAc$	270	2	-
(v)	Ethanol/Boric acid + $NaOAc$	361	4	-
(B) Aglycone				
(i)	Ethanol	255,369	-	-
(ii)	Ethanol/AlCl3	415	-	46
(iii)	Ethanol/$NaOAc$	255	-	-
(iv)	Ethanol/Boric acid + $NaOAc$	368	-	-
(C) Aglycone of methylated glycoside				
(i)	Ethanol	253,362	-	-
(ii)	Ethanol/AlCl3	421	59	-
(iii)	Ethanol/$NaOAc$	252 -	-	-
(IV)	Ethanol/Naoac	260,403	-	41

IV.30 I.R. SPECTRUM

The prominent peaks in the I.R. spectrum of the glycoside are at 3350 cm^{-1}, 2850 cm^{-1}, 1604 cm^{-1}, 1500 cm^{-1}, 1450 cm^{-1}, 1185 cm^{-1}, 1150 cm^{-1}, 1130 cm^{-1}, and 835 -810 cm^{-1}.

SECTION - C

IV.31 CHEMICAL STUDY OF THE COMPOUND (F)

An Orange-ish-red compound (F), m.p. 300°C was isolated from the acetone extract of water soluble fraction of Gardenia gummifera Linn. as described on page 131. The compound having molecular formula $C_{21}H_{21}O_{10}$, was shown to be a single entry by paper chromatography.

The ethanolic solution of the compound gave following reactions :

(i) It gave positive Molisch's test showing the presence of sugar moiety in the molecule.

(ii) It did not reduce Fehling's solution.

(iii) It also did not respond to the positive test with aniline hydrogen phthalate[45].

The above reaction ((ii) and (iii) specific for free aldehyde group in the sugars), suggesting that reducing group of the sugar moiety is not freely involved in linking end.

When the ethanolic solution of the compound (F) was heated for 5 minutes on boiling water-bath and it was extracted with amylalcohal, produced a violet colour with aqueous sodium acetate, showing it to be an anthocyanidin, therefore the original Compound(F) shall be an anthocyanin. On acid hydrolysis of glycoside(F) an aglycone and sugar moiety was obtained.

IV.32 AGLYCONE

The R_f values of the aglycone were found 0.71 and 0.52 in acetic acid: concentrated hydrochloric acid: water (30:3:10 v/v)

system and (5:1:5 v/v) system respectivaly. The aglycone of the compound (F), gave bluish red colour of the corresponding coloured base, with the treatment of sodium acetate and sodium carbonate. This colour change from bluish red to red was found quite stable in sodium acetate, solution. The stability in coloured base is well known in the case of flavylium salts having free hydroxyl groups at -3, and -5 positions. The aglycone did not respond positive Ferric chloride test, indicating, thereby the absence of any catechol unit in the molecule.

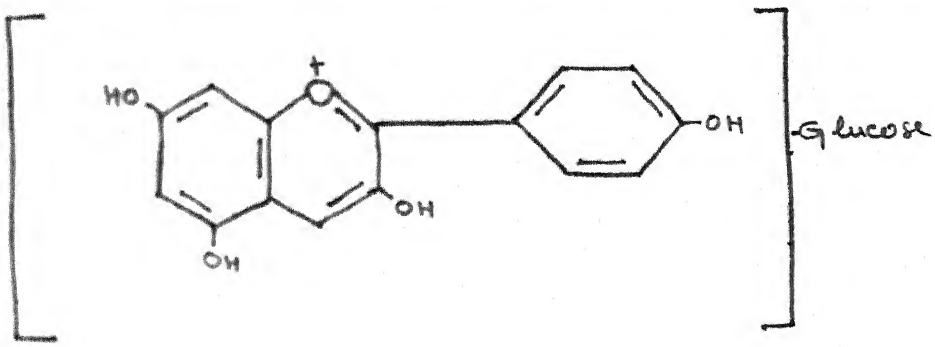
The λ max of aglycone of the compound, in ethanolic hydrochloric acid solution was found to be 530 nm and in methanolic hydrochloric acid 520 nm. According to Harborne[47] this shift from solvent to solvent corresponds to the pelargonidin. But the compound(F), gave λ max 513 nm[67] in methanolic hydrochloric acid. In case of anthocyanins it is generally known that substitution of one hydroxyl group lowers the λ max by 5 nm[68]. The aglycone did not show any bathochromic shift of the absorption maxima in the visible region even with aluminium chloride. This confirms the absence of any catechol unit in the molecule[57].

From the foregoing discussion it appears that the compound (F) is a pelargonidin derivative.

IV.33 IDENTIFICATION OF SUGAR

The sugar was identified by paper chromatography in n-Butanol: acetic acid: water (4:1:5 v/v) system, which revealed a single spot with Rf value 0.18. It formed an osazone with the treatment of Phenyl hydrazine as usual method. This suggests the presence of glucose. It was further confirmed by co-chromatography with an authentic sample.

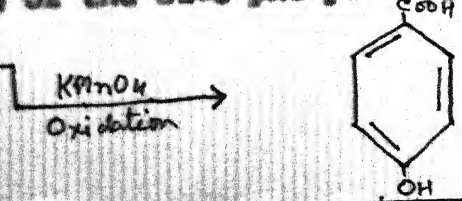
From the above evidence it is clear that the compound(F) is a pelargonidin derivative with glucose unit attached as sugar moiety in the glycoside linkage and can be represented as follows:



IV.34 POSITION OF GLYCOSIDIC LINKAGE

The position of glycosidic linkage in the glycoside was determined on the basis of distinct absorption maxima in the visible region. The addition of sugar residue to the 3-position of the anthocyanidin molecule causes a large colour shift towards shorter wavelengths, but further addition of sugar to the 5-position has little effect on λ max so that there is very little difference in colour between 3-glycoside and 3,5-diglycoside. Pelargonidin 3-glycoside has λ max 507 nm in methanolic hydrochloric acid and its 3,5-diglucoside has λ max 504 nm. But the present glycoside has λ max, 513 nm which indicates that sugar moiety is attached at -5 position[52].

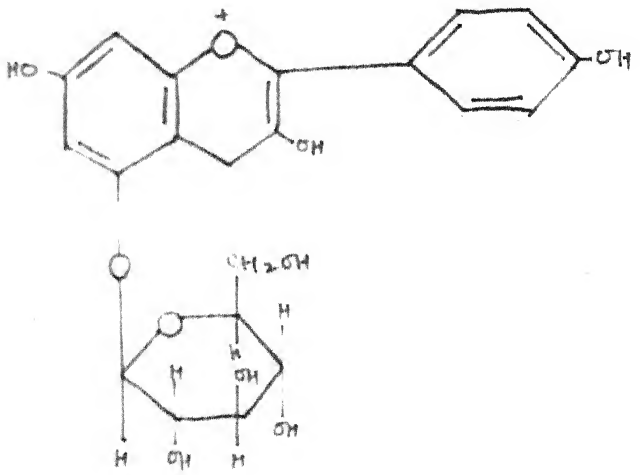
It is further confirmed on the basis of biogenetic considerations and negative colour test with vanillin hydrochloric acid reagent[69]. When compound (F) was subjected to neutral potassium permanganate oxidation, p-hydroxy benzoic acid was obtained. This confirmed the structure of the side phenyl ring (B) of the molecule compound (F)



The periodate oxidation studies of the glycoside showed that sugar is present as monosaccharide with the consumption of two moles of periodate with the liberation of one mole of formic acid per mole of the glycoside, from which it may be concluded that the glucose is present in the pyranose form. The pyranose structure of the sugar is also confirmed by the presence of medium peak in the region of 845 - 820^{cm-} in I.R. spectrum of the compound[73,74,87].

The glycoside was completely hydrolysed with the emulsin, which is specific for β-linkage. Hence, the nature of linkage between glucose and aglycone moiety seems to be β-linkage.

The above all evidences, suggest that the compound (F), is Pelargonidin 5-β-glucopyranoside and may be represented as below:



Pelargonidin - 5- β -glucopyranoside Compound (F)

EXPERIMENTAL

V.35 ISOLATION AND PURIFICATION

The compound (F), m.p. 300°C, was isolated from the fruits of Gardenia gummifera Linn, as described on page 131. The compound has repeatedly crystallised from methanol, till a pure product was obtained.

V.36 HOMOGENIETY OF THE COMPOUND

The homogeniety of the compound was checked by paper chromatography on Whatman No.1 filter paper using following solvent systems:

(i) n-Butanol: Acetic acid: Water (4:1:5 v/v)

(ii) Acetic acid :concentrated hydrochloric acid:Water(30:3:10V/V

(iii) Acetic acid: concentrated hydrochloric acid:Water(5:1:5 v/v

In each case a single spot was observed.

ELEMENTAL ANALYSIS

Found	Calculated for $C_{21}H_{21}C_{10}$
C = 54.52%	C = 53.8%
H = 3.95 %	H = 4.48%

The glycoside (50 mg) was dissolved in small quantity of 5% ethanolic hydrochloric acid(50 ml) for half an hour. The orange-red solution was diluted with water (50 ml) filtered off and the filtrate was extracted thrice with small amount of amylalcohol. The anthocyanidin was transferred in 1% aqueous hydrochloric acid solution with excess of Petroleum ether (40-60°). The acid layer was washed with light petrol and with benzene repeatedly, excess of concentrated hydrochloric acid was added in it, when a deep crimson coloured solid(aglycone) crystallised out. The filtrate obtained after removal of the aglycone was neutralised with barium carbonate, filtered and concentrated under reduced pressure to a syrupy mass.

IV.37 CHROMATOGRAPHY OF AGLYCONE

The purity of the aglycone was checked on Whatman No.1 filter paper when a single spot was observed in each case using following solvent systems :

(i) Acetic acid- concentrated hydrochloric acid - Water (30:3:10 v/v) system Rf .71

(ii) Acetic acid - concentrated hydrochloric acid - Water (5:1:5 v/v) Rf .52

These Rf values, corresponding to the Pelargenidin[75].

IV.38 IDENTIFICATION OF SUGAR

The syrupy mass obtained by the hydrolysis of the glycoside, reduced the Fehling's solution and it formed an osazone having m.p. 190° when treated with phenyl hydrazine reagent. The rf value of the sugar was 0.18 in n-Butanol:acetic:Water(4:1:5) v/v) system, which corresponds to D-glucose. The identity of the sugar was further confirmed by the co-chromatography with an authentic sample.

Potassium permanganate oxidation

The glycoside(F),(30 mg) was refluxed with aqueous potassium permanganate solution (20 ml) on a water-bath for four hours. The reaction mixture was cooled and excess of manganese dioxide removed by adding sodium bisulphite to it. The reaction mixture then was extracted with ether and it was shaken well with saturated aqueous sodium bicarbonate. This was acidified with hydrochloric acid and then extracted with ether. On concentration the ether extract gave p-hydroxy benzoic acid, which confirmed by mixed m.p. and paper co-chromatography with an authentic sample. The purified p-hydroxy benzoic acid had m.p. 210 C. (Lit. m.p. 213°C)[56].

The Rf value 0.86 in butanol:pyridine:aqueous sodium chloride (1:1:2) saturated system[76], which corresponded to p-hydroxy benzoic acid.

IV.39 PERIODATE OXIDATION

The glycoside (30 mg), was dissolved in(20 ml) aldehyde free ethanol (90%) and to it saturated sodium metaperiodate solution (20 ml) in same ethanol was added and made upto 50 ml solution in a measuring flask. After 40 hours, (5 ml) aliquots were taken out from the reaction mixtures. The periodate consumption was estimated by titrating against standard hypo solution and liberated formic acid by titrating against standardised sodium hydroxide solution due to the method of Jones etal[51]. Molecular weight of the compound (F) = 468.70.

For 5 ml of the solution	
0.011N sodium thiosulphate(hypo) consumed	= 2.3 ml
and 0.011 sodium hydroxide consumed	= 0.58 ml
For each mole of the glycoside	
moles of formic acid liberated	= 1.1
moles of periodate consumed	= 2.1

IV.40 ENZYMIC HYDROLYSIS

The glycoside(20 mg) was dissolved in aqueous ethanol(20 ml) and emulsin solution (25 ml) was added and the mixture was kept at room temperature for four days. Then the mixture was extracted with amyl alcohal. The aqueous layer was concentrated to a syrup. The paper chromatography of syrup in n-butanol:acetic acid:water (4:1:5 v/v) revealed the presence of a single spot, R_g 0.18, corresponding to glucose.

IV.41 ABSORPTION SPECTRUM AND VISIBLE SPECTRA OF THE COMPOUND (F)

All the measurment were taken using Beckmann model D.V. spectrophotometer and 1% methanolic hydrochloric acid and 1% ethanolic hydrochloric acid as medium.

Solution and reagent	max(nm)	Shift
(A) GLYCOSIDE (F)		
(i) A+methanolic hydrochloric acid[88]	513	-
(ii) A+ethanolic hydrochloric acid	523	-

(B) AGLYCONE	max of the compound in nm	max on addition of AlCl3 solution
(i) B+Me-OH.HCl[89]	520	No shift
(ii) B+Et-OH.HCl	530	No shift

IV.42 I R SPECTRUM OF COMPOUND (F)

The following prominent peaks (cm^{-1}) were observed in the IR spectrum of the glycoside :

1620 cm^{-1}, 1550 cm^{-1}, 1500 cm^{-1}, 1440 cm^{-1}, 1380cm^{-1}, 1287 cm^{-1}, 845-820 cm^{-1}.

REFERENCES

1. Duthie, J.F., 'Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalyan tracts', Vol.I , P.387, (1960), Copyright by Government of India.
2. Chopra, R.N. and I.C., and Nayer S.L. 'Glossary of Indian Medicinal Plants', Page No. 123 (1956).
3. Howes, F.N. 'A Dictionary of useful Everyday Plants and their common names', Page No. 81, (1974).
4. James Y.P., Chan. U.S. 3,067, 103 Dec.4, 1962, Appl.July 22,(1960)
5. Kako Honsha K.K. Jpn. Kokai Tokyo Koho 80, 108, 464, 20, Aug. (1980), Appl. 79 116, 456, 15 Feb.(1979).
6. Kim, Gang Man. Sunyu Konghak, Holji, 13 (3), 129-32(1976)(Korean).
7. Mikami, Yoichiro, Yajima, Izuma, Izumi (Hasegawa, T., Co.,(Ltd.) Jpn. Kokai, Tokyo Koho 79 96,532 (cl.C09B 61/00)31 July.1979, Appl.78/21 648,1 7 Jan.(1978).
8. Yu, Ju-Hyun., Yoo, Seung-Kon., Yang, Ryung, Hanguk Siktpum Hwahakhoe chi(1975), 7 (1), 30-6 (Eng.).
9. Asai, L.T. and Nakamura, M. Bot Mag. Tokyo 33 , 70-1(1919).
10. Munesada, L.T., J. Pharm. Soc. Japan. No. 486, 666-71(1922).
11. Kozo Hayashi; Proc. Imp. Acad.(Tokyo) 20 , 311-17 (1944) (Germ.).
12. Makino, Takeda, Yoshio; Nishimura, Hiroshi, Kudota, Osamu; Inouye, Hiroyuki; Chem. Pharm. Bull, 24 (11), 2644-6 (1976) (Eng.).
13. Inouye, Hiroyuki; Takeda, Yoshio, Shaito, Setsuo; Nishimura, Hiroshi, Sakuragi, Ryako; Yakugaku Zasshi, 94(5),577-86(1974)(Japan.
14. Veda, Shinichi; Kobayashi, Koji,; Muramatsu, Takeharu; Inouye, Hiroyuki (Fac. Pharm. Sci., Kyoto Univ. Kyoto, Japan). Planta. Med., 41(2), 186-91, (1981). (Eng.).
15. Matsumoto, Mitsuo; Uehara, Yusaku, Japan. Kokai 7829, 965, 20 Mar (1978), Appl. 76/103, 963, 31 Aug.(1976).

16. Kozo, Hayashi, Tachiko Iaaka, and Gen Suzushi; Misc. Repts. Research, Inst. Nat. Resources No.17-18, 33-42 (1950).

17. Inouye, Hiroyuki; Takeda, Yoshio; Nishimura, Hiroshi. Phytochem., 13(10), 2219-24, (1974) (Eng.).

18. Endo, Tohru; Taguchi, Heihachiro, Chem. Pharm. Bull, 21(12), 2684-8 (1973), (Eng.).

19. Inouye, Hiroyuki, Saito, Setsuo; Tguchi, Heihachiro, Endo, Tohru, Tetrahedron Lett., 28, 2347-50 (1969) (Germ.).

20. Kasuhara, Nobuo; Suzuki, Shinji; Shioda, Asao., Japan Kokai, (1976), Appl. 74/143, 059, 14 Dec. (1974).

21. Inouye, Hiroyuki, Saito, Setsuo, Shingu, Tetsuro, Tetrahedron Lett., (41), 3581-4, (1970) (Germ)

22. Endo, Tohru; Taguchi, Heihachiro; Chem. Pharm. Bull., 18(5), 1066-7 (1970) (Eng.).

23. Yung-chi, Chun, Yao Hsueh Hsuch Pao,11 (5) ,342-5(1964).

24. Chatterjee, Mrs. A., Saha, S.K.; Battacharya, S.,(Dep. Pure. Chem. Univ. Call. Sci., Calcutta, 700009 India, Indian J.Chem., Sect. B. 19 B(5), 421-2 (1980) (Eng.).

25. Chhabra, S.C., Gupta, S.R.; Sharma, N.D. (Dep. Chem. Univ. Delhi, Delhi, India), Phytochem. 16(3) ,399 (1977) (Eng.).

26. Krishnamurti, M., Seshadri, Tiruvenkata, R., Sharma, N.D., Indian, J.Chem. 9(2), 189-90 (1971), (Eng.).

27. Rao, A.V.,Rama; Venkataraman, Krishnaswami, Chakrabarti, P.; Sanyal, A.K.; Bose, P.K., Indian J. Chem.,8(5), 398-400(1970)(Eng

28. Kumari, Durga, Gupta, S.R; Sharma, N.D., Indian J. Chem. Sect.B. 17(B 2) 181-2 (1979) (Eng.).

29. Dutta, M.K., Ganguly, S.N., and Bhattacharya, A.N., J.Indian. Chem. 43 (5), 380 (1966) (Eng.).

30. Reddy, G.G.S., Ranganwami, S.; Sundar, R., (Dep. Chem. Univ. Delhi, Delhi India), Planta Med., 32 (3),206-11 (1977) (Eng.)

31. Joshi, Krishna, C., Singh. P., Pardasani, R.T.,(Dep.Chem. Univ. Rajasthan, Jaipur, India), J. Indian. Chem.Soc.56 (3), 327-8 (1979) (Eng.).

32. Forster, M.O., and Keshaviah, Aswath Narain Rao, J. Chem. Soc., 127, 2176, (1925) (Eng.).

33. Reddy, G.C.S. Ayengar, K.N.N., Rangaswami, S., Phytochem., 12(7), 1831 (1973) (Eng.).

34. Gunatilaka, A.A., Leslie, Sirimanne, Sarath, R.,Sotheeswaran, Subramaniamm, Nakanishi, Tsufomi, J.Chem.Res.Synop.(7), 216-17 (1979) (Eng.).

35. Delaude, C.; Kapundu, Mpuza Bull. Soc. R. Sci., Liege, 44-56, 493-4 (1975) (Fr.).

36. Govindachari, Tuticorin R., Jadhav, S.J., Joshi, Balwant, S., Kamal, Venkatesh. N., Mohamed, P.A., Parthasarathy, P.C., Patankar, S.J.; Prakashi, D., Rane, D.F., Viswanathan, N., India, J.Chem. 7 (3), 308-10 (1979)(Eng.).

37. Reddy, G.C.S., Ayengar, K.N.N.; Rangaswami, S., Phytochem., 14 (1), 307 (1975), (Eng.).

38. Reddy, G.C.S.; Ayengar, K.N.N.; Rangaswami, S., India J.Chem., 13 (7), 749-50 (1975) (Eng.).

39. Aidia Ochrolenca and Aidiamicrantha, Delaude,C., Bull, Soc.R. Sci., Liege, 43 (3/4) 257-9(1974) (Fr.).

40. J. Pharmacol. 4, 64-8, (1954) E., Ibid 3, 1(1953).

41. Aburada, Masaki,; Sasaki, Hiroshi, Harada, Masatoshi, Yakugaku Zasshi, 96(2), 147-53 (1976), (Japan).

42. Darwis Amar and A. Grevenstukl. Geneeskuna Tijdschar.Nederland India,76, 1948-84 (1936).

43. Shinoda, J.; J. Pharm. Soc.(Japan), 48, 214 (1928).

44. Murti, V.V.S.,Rajagopalan, S. and Row, L.R.; Proc.Ind.Acad. Sci. 34 , 319-23 (1951).

45. Asahina, Y; and Inubuse, M.; Ber., 61, 1946 (1928).

46. Briggs, L.H. and Locker, R.H.; J. Chem. Soc.,3136 (1951).

47. Geissman, T.A.; 'Modern Methods of Plant Analysis' ed by Peach, K. and Tracey, M.V, Springer-Verlag, Berlin,Vol.III, 450 (1955).

48. Venkataraman, K, In ' Progress in the chemistry of Organic Natural Products' Ed. by Zeichmeister, California Institute of Technology, Padasena, Vol. 17.

49. Sundsen, A.B.; Pharm. Acta. Helv; 34, 9 (1959).

50. Asahina, Y and Inbuse, M; Ber. 64 1256 (1931).

51. Jurd, L. and Horowitz, R.M.; J. Org. Chem., 22 1618 (1957).

52. Jurd, L.; 'The chemistry of Flavonoids compounds', ed. by T.A. Geissman, Pergamon Press, Oxford, P. 107 (1962).

53. Nordstron, C.G. and Swain, T., J.Chem. Soc.,2764 . (1953).

54. Mansfield, G.H., Swain, T and Nordstron, C.G., Nature,172,23(1953)

55. Willstaller, R. and Mallison, H., Anan. 408, 40 (1915).

56. Dimorth, O. and Faust, T.; Ber., 54 , 3030, (1921).

57. Morhammer, L. and Mensel, R.; Arch. Pharm. Berl., 286, 425 (1953); 288 , 315 (1955).

58. Wilson, C.W., 'A study of the Boric Acid colour Reactions of Flavone Derivatives', J. Amer. Chem. Soc.,61, 2303, (1939).

59. Horowitz, R.M., J. Am. Chem.Soc.,79, 6561, (1957).

60. Swain, T., Chem. and Ind., 1480, (1954).

61. Millis, W.E. and Vrbach, G., Nature, 182, 657, (1958).

62. George, T.B., Douglas,C.D. and Wander, S.M., Anal.Chem., 23 , 1982 (1951).

63. Wisenberger, Mikro Chemic., 33 , 51, (1947).

64. Belcher, R. and Godbert, A.L.; In semi-micro Quantitative organic Analysis', P. 164, Longmann-Green and Co.,New York,IInd Ed(1954).

65. Belcher,R., Fildes, J.E. and Nutten, A.J., Analytical Chem. Acta. 13, 16 (1955).

66. Shimizu, M., J.Pharm. Soc.(Japan), 1339,71 , (1957).

67. Douglus, C.D., Morris, Q.L. and Wender, S.H, J. Amer.Chem. Soc. 4023, 73 (1951).

68. Horowitz, R.M., J. Org. Chem. 1733, 22, (1957).

69.(a) Dean, F.M., In 'Naturally Occuring Oxygenring Compound', P.288, Butterworths, London (1963).

(b) Wilson, C.W., J.Amer. Chem. Soc., 2303, 61 (1939).

70. Hill, R.D. and Meakins, G.D., J. Chem. Soc. 760 (1958).

71. Bell, J.V., Heisler, J., Tann-enbaum, H. and Goldenson, J., Anal. Chem. 1720, 25 (1953).

72. Ory. M.A., Anal. Chem., 509, 32, (1960).

73. Wagner, H., In 'Methods in Poly Phenol Chemistry', edited by J.B. Pridham, P. 38, Pergamon Press,Oxford, (1964).

74. Shaw, B.L and Simpson, T.H., J.Chem.Soc., 5027, (1952).

75. Jurd, L. and Horowitz, R.M., J.Org.chem. 1395, 21 ,(1956).

76. Feighl, F. In 'Spot tests in Organic chemistry', Elsevier Publishing Company, London (1960) P. 466.

77. Gage, T.B., Doughlass, C.D. and Wender, S.A., Anal.Chem. 1958, 23 , (1951).

78. Jurd, L., Arch. Bio.Chem. Bio Phys., 376, 63 , (1956).

79. Hough, L., J. C hem. Soc., 1702, (1950).

80. Ayako, M. and Shunkeda, Nippon Nogei Kagaku Kaishi, Japan, 317, 39 (8), (1965).

81. Jurd, L. and Ralle, L.A., J. Amer. Chem. Soc.,5227,80(1958).

82. Segal, L. O' Connor, R.T, and Eggerton, F.V., J. Amer.Chem. Soc., 2807, 82 , (1960).

83. Bunket, J.C. and Badger, R.M., J.Amer.Chem.Soc,4397,72(1950).

84. Heilbron, I. and Bunbury, H.M. In 'Dictionary of Organic Compounds' , Vol. II, P. 269.

85. Hirst, E.L. and Jones, J.K.N. J.Chem.Soc.,1659(1949).

86. Richter, D. J. Chem. Soc., 1702 (1950).

87. Simpson, T.H. and Garden, L., J. Chem. Soc.,4638,(1952).

88. Jurd, L., J. Amer.Chem. Soc.,80 , 5531, (1958).

89. Hochhammer, L. and Hansel, R., Arch. Pharm., Ber.87,287,(1954).